EXHIBIT A

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

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PHILLIP RACIES, on behalf of :
Himself and All Others :
Similarly Situated, :
Plaintiffs, : Case No.
vs. : 3:15-CV-00292 HSG
QUINCY BIOSCIENCE, LLC, a :
Wisconsin limited liability :
company, :
Defendant. :

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Toronto, Ontario, Canada
Friday, October 16, 2015

Videotaped Deposition of:

DR. RICHARD BAZINET

the witness, called for examination by counsel for the Defendant, pursuant to notice and agreement, commencing at 10:08 a.m., at Toronto Court Reporters, 65 Queen Street West, Suite 1410, Toronto, before Virlana Kardash, RPR, CSR, Commissioner of Oaths, when were present on behalf of the respective parties:

11:23 cross the BBB or proteins that would be taken by mouth.

Q How about peptides? Can they cross the BBB?

11:23 A Yes, some peptides can cross the BBB.

Q Any peptides in particular that you can think of, off the top of your head? We may get into a couple later on.

A Oh, there's -- the names are slipping right

11:23 now. I think I referred to a study by Stanley Rapoport in my report that would have a list of known peptides that cross the BBB.

Q That was Rapoport?

A Yes.

11:24 Q But it's your opinion that no dietary proteins cross the BBB; is that right?

A Correct.

Q All right. Let's take a break. We've been going almost an hour and a half.

11:24 Is that okay, Stewart?

MR. WELTMAN: Oh, yes. Sure.

BY MR. SIMON:

Q Is that okay with you?

A Yes.

11:24 VIDEOGRAPHER: This marks the end of

Q No. I don't want you to do that. Any that readily come to mind? 12:00

A Not any that readily come to mind.

Q Any peptides that you can think of that can have an effect on memory without entering the BBB? 12:00

MR. WELTMAN: Objection. Vague as to the term "effect."

THE WITNESS: So some peptides -- memory? Can you define "memory"? Memory is a big term. It has a lot of sub-definitions. 12:00

BY MR. SIMON:

Q Maybe I can later. I can't right now. I'm not an expert on memory. But you can tell me what you understand the word "memory" to mean. Can you do that? 12:00

A There's lots of definitions of memory. So there's molecular memory. There's reflex memories. There's remembering your name. There's short-term memories. There's long-term memories. There's synaptic plasticity. 12:01

So some of those things could be affected by molecules that are not crossing the blood-brain barrier, yes.

Q I was asking specifically about peptides. Sorry. 12:01

A Peptides? 12:01

Q We already established that molecules can have an effect. I wanted to know about peptides in particular.

A It's a complex story, but yes, peptides could affect memory very indirectly without crossing the BBB. 12:01

Q Can they have an effect -- instead of saying "effect," how about we stick with the opinion here. I want to talk about brain function. The opinion is that Prevagen cannot improve or support healthy brain function. 12:01

So I want to know whether or not there are any molecules that could support healthy brain function that don't cross the BBB. 12:02

A Sorry. Sorry. I think -- was the term "molecules" used again?

Q I used "molecules" this time.

A Okay. And you meant to use molecules?

Q Yes. 12:02

A Are there any molecules -- I'm sorry. Could you repeat the question?

Q Sure. Are you aware of any molecules that can support healthy brain function without crossing the BBB? 12:02

01:34 A So there was a study on dogs.
Q What did that study on dogs show?
A The study on dogs measured AQ in the blood or attempted to measure AQ in the blood and the
01:35 cerebrospinal fluid, which would be considered a marker of entry into the brain. And the study's got issues.
But if you get around those issues and just look at the data, it shows they couldn't detect it in the
01:35 blood or the brain.
Q Any other studies that you're aware of that show that AQ doesn't get in the blood?
A So like I said, the body of literature which allows for extrapolation that says all dietary
01:35 proteins do not get into the blood is part of that evidence.
Q When you say all dietary proteins do not get into the blood, do you mean the protein as a whole as ingested or any aspect of that digested protein?
01:36 A Proteins are broken down into amino acids and some small peptides in some cases. And those are how we absorb proteins, actually. So you've got to be careful with the words because you'll see the words dietary protein absorption.
01:36 And usually what those studies are referring to

effect. 01:42

Q Okay. And that distinction you're making, it may lessen the digestion to a certain extent but not in a meaningful way; is that what you mean?

MR. WELTMAN: Objection. Vague. 01:42

BY MR. SIMON:

Q Go ahead.

A No. What I'm saying is digestion will -- you know, this is common -- will usually begin in the stomach. And if you encapsulate something, you can 01:42 start digestion later. But I wouldn't perceive the amount -- it's not like if you delay something a little bit, it translates into less being digested.

It just changes the dynamics of the digestion, but they're still digested. 01:42

Q Could cellulose or rice flour, could that potentially prevent digestion of AQ within the stomach?

MR. WELTMAN: Objection. Incomplete hypothetical. 01:43

THE WITNESS: So it couldn't prevent, but depending on the matrix, it could delay.

BY MR. SIMON:

Q So it could decrease the speed at which AQ is digested in the stomach; is that right? 01:43

01:49
BY MR. SIMON:
Q Now I'm asking whether it's entirely digested into single amino acids.
A No. Dietary proteins aren't all entirely digested into single amino acids.
01:49
Q Do you have evidence that AQ is entirely digested into single amino acids?
A No.
Q So we talked about the possible -- go ahead.
01:49
A Sorry. Sorry. It's -- there is a piece of evidence provided by Quincy that I don't think I used in the first part of my report where they tried to digest an assay. And they showed that it was --
01:49
Q That's the allergenicity study?
A Yes, it would be.
Q We'll look at that.
A Yes.
Q So that's the one piece of evidence that you can think of that supports the idea that AQ is entirely digested into single amino acids?
01:50
A No. So proteins are digested -- let's be clear on this -- to predominantly amino acids and some small peptides. There probably is a case of a protein that doesn't give a peptide.
01:50

01:50 But we have to be very careful with that because in science you can only measure things so far, and you get down to some weird unit that we haven't been able to see at that level.

01:50 Q Do you believe that the document that Quincy provided supports the proposition that AQ is entirely digested into single amino acids? You don't agree with that; right? You don't agree with that proposition because you believe that all proteins are

01:50 digested into predominantly single amino acids and small peptides?

A Unfortunately, the Quincy study didn't measure peptides. It's almost impossible to do. There's lots of them. So it's consistent with that

01:51 ideas, but I would expect there to be some peptides at some level in there, yes.

Q Now, we were discussing the possible mixture of apoaequorin with rice flour cellulose. We talked about a potential delay in digestion that might

01:51 occur in the stomach?

A It could increase the digestion also. It could alter. It could increase the digestion. It could decrease it. Lots of times when you mix foods with other foods, you can improve your digestion.

01:51 Q Do you have any opinion as to whether white

process up. 01:55

Q It would speed the process of digestion of AQ in the stomach?

A It would speed -- depending on how much acetic acid is in there, it would speed up the denaturation, which would speed up the digestion. 01:56

Q How does denaturation speed up digestion?

A When proteins hit acids -- so proteins -- and I'm sorry. I have to use a hand gesture here. They have complicated three dimensional structures. And the proteins -- digestion proteins can get at that a bit. 01:56

But it helps when they unfold flat. The surface areas increase; so you can speed that up. And acids help unfold proteins. 01:56

Q Have you personally run any studies on whether any of the mixtures with AQ in the Prevagen product would have any effect on digestion of AQ in the stomach?

A Have I personally run those studies? I haven't. 01:56

Q Do you know of any specific studies that consider the mixture of white rice flour, cellulose, salt, magnesium stearate, acetic acid with apoaequorin, the effect that mixture would have on the 01:57

digestion of AQ -- 01:57
A So these compounds that you discuss are all very common in foods, and foods don't have major effects on the absorption and digestion in terms of the completeness. So yes, there's a lot of work 01:57 examining these things at much higher doses than would be in these things.
And there's no measurable effect on the net effect.
Q But you're not aware of any study that 01:57 deals specifically with those specific substances and AQ; correct?
A No.
Q You'd agree that I'm right -- I asked correct, and you said no. So do you disagree with 01:57 what I said?
A So I agree that there are no published studies that have added magnesium stearate, I believe you said acetic acid, and white rice flour on the -- call it metabolism of AQ, yes. 01:58
Q Based on your expertise, do you believe there to be a difference between in vitro digestion of a purified protein in a test tube and ingestion and digestion of foods in the stomach of a living organism? 01:58

A Assay designed to -- there's so many. There's all kinds of papers on this. There's a lot of different models to test digestion, absorption. And the resistance is kind of one type of one of those assays. 02:04 02:04

Q Have you studied the difference between those two types of assays?

A So there's not two types. There's -- one would have many types, many variabilities, many differences between them. And yes, I've looked at some of those differences. 02:05

Q All right. Let's look at the digestion study.

MR. WELTMAN: Let's go off the record.

VIDEOGRAPHER: This marks the end of media No. 2 in the deposition of Dr. Richard Bazinet. We're going off the record at 2:05 p.m. 02:05

(Recess from 2:05 p.m. to 2:14 p.m.)

VIDEOGRAPHER: Here begins media No. 3 in the deposition of Dr. Richard Bazinet. We're back on the record at 2:14 p.m. 02:14

BY MR. SIMON:

Q Are you aware of any reports showing pepsin resistent proteins?

A I've definitely seen reports of pepsin 02:14

resistent proteins, yes. 02:14
Q Do you have any knowledge as to whether AQ is a pepsin resistent protein?
A So the only -- do I have any knowledge? Can you repeat the question, sir? 02:15
Q Yes. Do you have any opinion as to whether AQ -- instead of knowledge, I'll use "opinion." Do you have any opinion as to whether AQ is a pepsin resistent protein?
A No. 02:15
Q Do you believe that ingested proteins can cause allergy?
A Yes.
Q And how does it work? We can use the peanut as an example. How does an allergy occur? 02:15
A So it's a very complicated question, and there are many ways it can occur. So the question how does it occur is a bit boxing me in to something that --
Q Are the proteins in a peanut that 02:16 exhibit -- how do I phrase that? Are proteins that cause allergy, those are those digested?
A Yes, they are digested. Well, you've got a funny little catch in this question here. Are proteins -- they can -- yes and no. 02:16

02:24 correct? And they're left for those other enzymes, those fancy words that I can't pronounce -- it's left for that to complete the digestion; is that correct?

MR. WELTMAN: I'll object to the word

02:24 "protein" as being vague in this context.

THE WITNESS: So are there other dietary proteins that are? Can you repeat the question?

BY MR. SIMON:

Q Yes. There are other dietary proteins that

02:24 are resistent to pepsin such that those other enzymes in the GI tract are needed to further break those proteins down; is that correct?

A So they're not needed. They're redundant. They're redundant. But they're not necessary for it.

02:25 You can still do it without them. But pepsin is an important one step of the process.

Q Sometimes like you just recently testified, things aren't fully digested in the stomach by pepsin; right?

02:25 A Correct.

Q And so further breakdown occurs in the GI tract with those other enzymes; right?

A Correct.

Q And that breakdown is either into smaller

02:25 peptides or single amino acids; right?

02:25 A Correct.

Q And a peptide can be broken down into smaller peptides; correct?

A Correct.

02:25 Q It doesn't necessarily have to be broken down into single amino acids in the GI tract; right?

A Correct.

Q And it can enter the blood as a peptide; right?

02:25 A Correct.

Q And we established earlier that peptides can cross the BBB; right?

A So --

Q Certain peptides can cross the BBB; right?

02:25 A Yes, we established that certain peptides can cross the BBB.

Q Let's look at your report here on paragraph 28, top of 28. "While there are some reports claiming that some proteins can get past

02:26 digestion and into the bloodstream," these are the reports you were talking about earlier?

And we'll actually look at them in a bit.

A Okay.

Q Are these the Quincy reports that you were

02:26 discussing earlier about proteins potentially getting

would not exist for apoaequorin." 04:52

Why could a receptor not exist for apoaequorin to allow it to be taken into the brain?

A So two things. First is it's not clear this molecule entered -- this polypeptide entered the brain through a receptor. Another paper which I cite says maybe it's got a receptor. That's using their wording here. 04:52

To evolve a receptor for a foreign molecule that would be this specific, which is just theoretical here, doesn't happen. So if you look at the writings which were cited in the Quincy report of Bill Partridge, he says that this does not exist for foreign proteins. 04:53

Q How about a peptide that can be broken down from the digestion of AQ? Could it be possible that there's a receptor that allows for that particular peptide to cross the BBB? 04:53

A So the AQ would be broken down -- so we've got to be very careful of the definition of peptides because they vary by chemistry and nutrition digestion. 04:53

Peptides in this sense are moderately large molecules, moderately large, somewhere around 20 to 50 amino acids. Okay? The polypeptides that we refer to 04:53

in digestion are two, three amino acids. It's just a nutritional distinction from a definition of a chemical term. 04:54

We use these terms a little differently in different fields. So the polypeptides, which are essentially dietary amino acids, dietary polypeptides, they're indistinguishable. So yes, I totally agree. The amino acids and the polypeptides of AQ behave just like the dietary proteins. There's no distinction. 04:54

Q And there are some receptors that can allow those peptides to cross the BBB; is that right? 04:54

A There might be some receptors that could help the larger peptides cross the BBB. The smaller ones, they're -- so for amino acids, yes, there are receptors that allow amino acids to cross the BBB. 04:54

Q How about peptides?

A Yes, there would be some.

Q Now, can smaller peptides cross the BBB by themselves; right?

A So yes, depending on how you define "can." 04:55
Again getting into the numbers that we were talking about for across the intestine, a small proportion -- so in the neighborhood of a millionth -- have been shown to cross the BBB.

And we think it's independent of a transporter. 04:55

scientifically nothing. 04:59

Absolutely -- so close to absolute -- like a mathematician/theoretical physicist using nothing. Trillions and trillions of times beyond what we call nothing in biology. So just put that into context. 04:59

Q Yes, that's great.

A Okay.

Q Now, my question was actually, bypassing all of that and injecting that, do you know for certain that AQ does not bind to the FGF 21 receptor? 05:00

A So the amino acid sequence, which I eyeballed, was published in the paper and is available for FGF don't show any similarities. So it seems almost impossible that it would bind to that receptor.

Q And you've compared the FGF receptor with the amino acid sequence of ATU? 05:00

A Yes, by eyeball. The amino acid sequence of ATU is published in the Moran study, portions of it. M-O-R-A-N. And nothing obvious jumped out.

Q Do you know whether AQ can bind to any BBB receptor assuming it's injected and gets to the blood-brain barrier? 05:00

A Any? So you have to recognize very few receptors actually transport -- are transporters; right? And so the -- 05:00

Page 245

could bind to a BBB receptor that could transport it across the BBB? 05:02

A So others in the literature have said very clearly that there are no known exceptions to this rule. There are no known exceptions. So in science, 05:02 we've always got to be a little careful if we get into this, "Is there a pink elephant in the room beside me" conversation.

So technically, I'm not in the room. I didn't look. So I've got to be open to the idea. But I can 05:02 say that there's no such thing as a pink elephant. It's never existed. It's never been reported. So I don't think there's one.

Q Do you know whether AQ can bind to any serum proteins that can then bind to a BBB receptor? 05:02

MR. WELTMAN: Objection. Are we still talking about an injection hypothetical?

MR. SIMON: Yes, we are.

THE WITNESS: So if you inject AQ, can it bind to a serum protein, and then that serum protein 05:02 can bind to a receptor? I don't know.

BY MR. SIMON:

Q You haven't ruled out that possibility in your report; right?

A Well, yes, I do because AQ would be 05:03

digested. 05:03

Q Other than the digestion, you have no other basis to say that AQ could not bind to a serum protein that can then bind to a BBB receptor to transport it -- 05:03

A I have no basis to say that it could or couldn't.

Q Do you know the size limit of protein transported across the BBB by receptor mediated transcytosis? 05:03

A No, I don't.

Q Do you know the largest size of a protein that can be transported across the BBB by a receptor?

MR. WELTMAN: Objection. Mischaracterizes his testimony. 05:03

THE WITNESS: No, I don't.

BY MR. SIMON:

Q What's the largest size of a protein that you know of that can be transported across the blood-brain barrier with a receptor? 05:03

A I don't know proteins by weights, just the most common ones. So I don't know their weights.

Q In paragraph 10 of your report, you mention that protein can lose its 3D structure.

A Yes. 05:04

05:15 are referring to peptides, not single amino acids, in these studies; is that right?

A It's a poor choice of words by the authors, but I would assume they're referring to small

05:15 peptides.

Q Do you have any basis to believe, based on your review of those reports, that they were referring to single amino acids?

A Not in the ones I cite here. But other

05:15 assays would run into this problem. This is an older literature that I think we can just dismiss because it's completely not valid.

Q In paragraph 11 of your report, it states here, "Significantly, the only notable exceptions are

05:16 some small peptides. The term 'peptide' refers to molecules of about 15 amino acids or less. And upon digestion, they consist predominantly of two or three amino acids, which apoaequorin is not."

"And even these small peptides with unique

05:16 properties are only absorbed at a rate of about 1 percent." Is it true that apoaequorin is digested into smaller peptides along with single amino acids?

A Yes. All dietary proteins. And this is an important point, putting this back into the

05:17 nutritional context. Apoaequorin is indistinguishable

peptides. 05:20

Q Can proteins vary in any respect with regard to how much of the protein is digested into single amino acids as opposed to peptides?

MR. WELTMAN: Objection. Vague as to the term "proteins." 05:20

THE WITNESS: You would expect they would, yes. Dietary proteins would cover an array of this, yes.

BY MR. SIMON: 05:20

Q Is there any protein you can think in particular that breaks down into more or less single amino acids when compared to other proteins?

MR. WELTMAN: Objection. Again vague as to the determine "protein." 05:20

THE WITNESS: I can't think of an example right now that is one that becomes absolutely 100 percent compared to one that's only 99 percent. I can't give you those numbers, no.

BY MR. SIMON: 05:20

Q I hear a lot that whey is supposed to be like the super protein. Is there any difference in terms of how whey is broken down in the body with regard to single amino acids as opposed to small peptides when compared to other protein? 05:21

05:21 A So yes, proteins contain different amino acids. And so whey can have effects on -- whey protein can be a little different than the casein proteins, which is a little different than the soy
05:21 protein, yes.

Q Does casein break down more or less in terms of single amino acids when compared to whey; do you know?

A I don't know that, no.

05:21 Q Do you know what peptides result from the breakdown of casein as opposed to whey?

A In the digestion?

Q In the digestion.

A So the way this works, it would be very
05:22 complicated. It would be all kinds. It would be more or less random combinations of the 20 amino acids.

Q You don't have any data that shows that apoaequorin does not enter the intestine as peptides; right?

05:22 MR. WELTMAN: Objection.

THE WITNESS: So as I said, all dietary proteins to some extent or the vast majority -- there would be millions; right -- would enter the intestine to some extent as a peptide.

05:22 BY MR. SIMON:
Q Let's talk a little bit about the characteristics that a molecule needs to have in order to cross the BBB.
05:22 A Yes.
Q Paragraph 52 spells that out. Paragraph 52 is long.
MR. WELTMAN: Is it 52?
BY MR. SIMON:
05:23 Q Yes. Let's look on page 22.
A Okay.
MR. WELTMAN: It starts on page 21, but that's okay.
BY MR. SIMON:
05:23 Q Yes, it does. I'm reading from page 22, lines 21 through 25.
A Yes.
Q You cite a document that states, "For a small molecule drug to cross the BBB in
05:23 pharmacologically significant amounts, the molecule must have the dual molecular characteristics of molecular mass under 400 to 500 dalton threshold and, two, high lipid solubility."
Do you agree with that statement?
05:23 A Yes.

05:23 Q There is an upper limit to molecular mass and high lipid solubility; is that right?
A Sorry? There's an upper limit?
Q Upper limit, yes.
05:24 A Maybe. I don't know.
Q Okay.
A I don't know.
Q How much is the molecular mass or molecular weight of a typical amino acid?
05:24 A I could do the exact arithmetic, but it would be -- nitrogen is 14. Call it 60 or something. Yes.
Q 60 would be the --
A They're all different; right? So I did
05:24 that roughly in my head. Somewhere around this.
Q So you're adding up the C terminus with the N terminus and the potential weight of amino acid; is that right?
A Yes, and the hydrogen. They all vary,
05:24 though. Every amino acid is different; so it's a ballpark.
Q So a tripeptide would be about 180 daltons; is that right?
A Yes, using that --
05:25 Q And a quadrapeptide would be about -- what

05:25 is that? 240 daltons?

A Yes.

Q How do you tell whether a peptide has high lipid solubility?

05:25 A You could tell by its amino acid structure a little bit. There's a few amino acids that are known to be lipid soluble, but it can sometimes depend on some other characteristics. The general point here is that amino acids are not very lipid soluble.

05:25 Amino acids -- we can get into this here. He's not referring to amino acids because they don't cross the blood-brain barrier in the definition he's using here. So it's kind of a little irrelevant in this context.

05:25 He's using a term "cross the BBB," and that's a little different than some of the stuff we've been talking about today.

Q Will a peptide have high lipid solubility if it's made only of hydrophobic amino acid residues?

05:26 A Not compared to the things he's talking about. So it's all a relative term. So they call amino acids lipophylic or water-loving. But compared to lipids, they're nowhere near; right? So it's a definition within that class of molecules.

05:26 I study lipids. Those are lipid soluble. So

05:40 wait long enough, does it cross? I'm not sure. In vivo, the amino acids use transporters to get across. It's not very controversial.

It's well described. It's how they cross.

05:40 Q How about an amino acid sequence; can that get across without a transporter?

A So some small polypeptides look like they can cross. Again, I want to make the point, when we say it looks like they can cross, I don't want you to

05:41 think about this as 10 on one side of the blood-brain barrier and then 10 in the brain.

We're talking about calculations that are done -- there's nothing controversial -- one 1 millionth crosses.

05:41 Q One 1 millionth --

A It varies.

Q -- of the peptides or the single amino acids? What are you talking about?

A So if you had a hundred thousand or a

05:41 million peptides on the one side, one may cross.

Q Got it.

A That's it. So when we're saying "can cross," I just don't want an image of all the ones just crossing. It's a small, small fraction that

05:41 cross. And when we're talking about can or cannot,

food-derived proteins and peptides have functional 05:44
benefits beyond nutrition?

A So there's a problem with the sentence. It's the word "and." So yes, peptides have benefits beyond nutritional. 05:44

Q But proteins don't necessarily have benefits beyond nutrition; is that right?

A So dietary proteins. This is the context of functional foods. Obviously, the synapses in the brain that are proteins have functions beyond 05:45 nutrition. But dietary proteins, you know, you can get examples.

So if you go with somebody who's protein deficient and then you give them back protein, obviously that has a functional benefit. But in the 05:45 context we're talking about here, proteins aren't what people are talking about here.

Q You wouldn't say that dietary proteins have no functional benefit -- right -- no functional benefit beyond nutrition; is that right? 05:45

A So --

MR. WELTMAN: Objection. Vague.

THE WITNESS: They probably have some benefits outside of nutrition.

BY MR. SIMON: 05:45

Q What kind of functions can a peptide have other than providing nutrition that you know of?

A So there's tons of peptides in the body that act as local hormones. Insulin is one we talked 05:46 about, a very important peptide.

Q And you would characterize insulin as a peptide, not a protein?

A Insulin is right on the cusp. It's just -- in my field, we tend to refer to it as the peptide 05:46 insulin. But it's right there. So the cut-off in chemistry, not nutrition, is usually about 50 amino acids. I think insulin is 53 or 56, from memory.

So it's right in that gray area. It's not an absolute rule at that. So that's what it is. It's 05:46 right on that. If you call it a protein, it's as small as you get. If you call it a peptide, it's at the larger end of the peptides.

Q You said there are many peptides within the body that provide functional benefit? 05:46

A Yes.

Q Can there be peptides that are derived from dietary sources that provide functional benefit within the body?

A So the way insulin works, so when you eat 05:47

meat, it would have insulin in it. The way this works 05:47
is it's not the insulin crossing into your -- getting through digestion and getting into your blood that releases insulin.

What you do is you would take those dietary 05:47
proteins, break them down into small peptides, amino acids, bring them in, recirculate those amino acids into the pancreas, into the synthesis of insulin. That's how dietary --

So all dietary, with the exception of amino 05:47
acids that you can make -- they're called the nonessential amino acids. So those ones can come from diet, or you can make them on your own. But the essential amino acids.

So every time you see an essential amino acid in 05:47
a protein, it had to come from the diet.

Q And I was talking specifically about peptides from dietary sources. Can they have functional benefit within the body beyond nutritional benefit? 05:48

MR. WELTMAN: Objection. Vague.

THE WITNESS: So yes, insulin. The example I have, insulin's benefits aren't related to -- it regulates carbohydrate metabolism. But insulin itself is not a -- 05:48

BY MR. SIMON: 05:48

Q Can a protein function as a signaling molecule?

A Yes.

Q Can a peptide function as a signaling 05:48 molecule?

A Yes.

Q And how does it function as a signaling molecule? By binding to the receptor; is that right?

A That's one way. 05:48

Q What's another way?

A Oh, there's all kinds. There's so many. So if a serotonin molecule, dopamine molecule, which is derived from an amino acid, binds to a dopamine receptor, it will lead to secondary messengers, which 05:48 would include phosphorylated proteins.

There's lots of secondary messenger proteins. There's countless amounts.

Q Now, if a signaling peptide or protein -- well, if a protein acts as a signaling molecule, 05:49 that's quite different than a piece of bread that's digested; right?

MR. WELTMAN: Objection. Vague.

THE WITNESS: Yes and no. The amino acids from the piece of bread that are digested can make 05:49

reason we use NMDA and not glutamate is glutamate does not cross the blood-brain barrier. It's one of those ones. So not very much if you put it on the neuron. That's why in that study we were injecting NMDA, not glutamate. 05:52 05:52

NMDA binds that receptor, the NMDA receptor. NMDA is a drug, more or less. It binds the receptor. But normally, glutamate would bind. But injecting glutamate doesn't work.

Q Could a single molecule of glutamate have an effect on a neuron? 05:52

A Not a measurable effect, I don't think. I guess you could draw a picture that says it binds to receptor and releases four calcium molecules. But you'd never be able to measure that in vivo. 05:53

Q How much calcium crosses the membrane of a neuron when it is excited, I guess is the word, by glutamate? Is that the proper term, "excited"?

A Activated. Excited.

Q Okay. 05:53

A I don't know the number.

Q You don't have an estimate as to how much calcium crosses the membrane of the neuron during its excitation by glutamate?

A No. 05:53

Q You've heard the term "cytotoxicity"? 05:53
A Yes.
Q If a neuron is stimulated too much, for example, by glutamate, it can loss function and even die; is that correct? 05:54
A Yes.
Q If something can tone down glutamate excitation which involves calcium, the neuron can be saved; is that correct?
A Yes. 05:54
Q And that's how the drug Memotine purportedly works in Alzheimer's patients to improve daily function; is that right?
A Oh, that's really, really, really controversial. But maybe. It's an idea behind it. 05:54
Q The makers of Memotine, the manufacturers, that's what they purport to happen; is that correct?
A I don't know what they put in their sheets. But that's a good idea.
Q Why do you say that's a good idea? 05:54
A Well, cytotoxicity kills neurons, and that's an active area of research. They're trying to stop neurons from dying from cytotoxicity, especially in stroke. Right? It's a big area.
Q If a peptide can carry out a 05:55

neuroprotective function, how much peptide would it take to block an NMDA receptor or calcium channel? 05:55

A So in theory, one molecule should block one receptor.

Q Do you know whether or not a calcium channel can be blocked by a ligand? L-I-G-A-N-D. 05:55

A Yes. So they should be ligands to block them, yes.

Q Can a ligand be a peptide?

A Yes. 05:55

Q Can it be a protein?

A The definition of ligand basically means anything that binds to anything. So yes.

Q Talking about these concepts, I believe we've established that a protein can have an effect on brain function without actually entering the brain; is that right? 05:56

A Yes. So most proteins are made within the brain and regulate brain function.

Q But I'm talking specifically about proteins that are outside of the brain. They don't have to enter the brain in order to have an effect on brain function; right? 05:56

MR. WELTMAN: Objection. Vague.

THE WITNESS: Proteins do not have to enter 05:56

the brain to have an effect on -- so there are -- yes, there are -- there are -- yes. 05:56

BY MR. SIMON:

Q And just so we're clear in light of counsel's objections -- I think he has a standing objection on this one -- you're aware that there are studies on human subjects reported in the documents Quincy produced and reviewed; is that right? 05:57

A Am I aware -- yes.

Q Let me finish with the follow-up question which goes to what -- his objection. You haven't reviewed those documents as part of your expert report; is that correct? 05:57

A No, that's not correct. I got the document, I looked through it, and I decided whether or not they related to body chemistry. And they didn't relate to body chemistry; so they're not in my report. 05:57

Q Okay. Do you recall seeing a double-blind placebo control study? 05:58

MR. WELTMAN: I'll object. It's outside the scope.

THE WITNESS: So it depends on how you define that, but there's something that appeared to be one. 05:58

AQ to bread and hotdogs. Now, you mentioned a person typically ingests about 75,000 milligrams of protein a day; right? 06:06

A I believe that number is in my report.

Q Can you tell me what's the suggested use for Prevagen? 06:06

A So there are a variety of doses. Ten, 20 milligrams. There may have been others.

Q Do you know what the suggested use for Prevagen is in terms of when to take Prevagen? 06:06

A No.

Q Does your report discuss the suggested use of Prevagen in terms of timing?

A No.

Q If someone takes a capsule first thing in the morning without food, that 10 milligrams of AQ is not mixed with 75,000 milligrams of other proteins, is it? 06:07

A It's worse than that but -- it's mixed with much more than that. It's mixed with your whole body. 06:07

Q If you take Prevagen in the morning, fasted, does that 10 milligrams, 20 milligrams, is that a bolus?

MR. WELTMAN: I'm sorry. I didn't hear that question, end of the question. Is that a what? 06:07

MR. SIMON: A bolus. 06:07
THE WITNESS: I wouldn't use that term. The term is not -- it's used in different contexts. You could use that term.
BY MR. SIMON: 06:07
Q When 10 milligrams is taken in a fasted state in the morning without food, that's the only protein intake for that person at that time; is that right?
A That's the only dietary protein intake at 06:08 that time if they're fasted.
Q And again, in your report you don't discuss that suggested use, do you?
A No.
Q How many amino acid residues are found in 06:08 proteins?
A There's all kinds of combinations. It goes on forever.
Q How about individual single amino acids? How many residues are there? I think you said 20 06:08 earlier; is that right?
A In proteins?
Q Yes.
A No. I didn't say 20. So it's generally considered with a little bit of gray area when you get 06:08

around the actual specific numbers. Somewhere over 50. 06:08

Q With 50 residues, how many different tripeptides can they form?

A I can't do that math in my head. It would be a ridiculous amount. It would be way more than the odds against you winning the lottery. 06:09

Q Would it?

A Yes. Ridiculous how many combination they could make. 06:09

Q Isn't it just --

A Depending on the lottery. But it would be a lot.

Q Wouldn't it just be 50 cubed?

A No, because you could have A plus A plus A, A plus B plus B. There's a lot of combinations. And then A plus B plus -- B plus A plus A is not the same as B -- there's a lot. There's a lot of combinations. It's not just 50 times three because that's assuming you can only have one combination. 06:09 06:10

Q So 50 cubed. So it would be 50 times 50 times 50?

A No, it would be more than that.

Q It would be more than that?

A I'd never finish drawing it here today. It 06:10

would be a lot. Because that assumption I think would 06:10
assume that amino acid ABB is the same as BBA, which
is not true. It's not like picking a number, and then
you pull the number out of your lottery ticket, and
the number is no longer in the pool. 06:10

The number is still in the pool. Right? So
that calculation doesn't work. I'd have to sit down
and draw it out. It would be a lot.

Q Quadrapeptides, that combination would be
even more than the tripeptides; right? 06:11

A No, it would be less.

Q Why is that?

A So the -- oh yes, sorry. It would be more.
I did it backwards in my head. Yes, every time you
add them, you get more potential combinations. 06:11

Q So let's say that 10 milligrams of Prevagen
can generate an unusual quadrapeptide that crosses
into blood and the brain. That peptide sequence may
or may not be found in other dietary proteins; is that
right? 06:11

MR. WELTMAN: Objection. Calls for
speculation.

THE WITNESS: It would be found in other
proteins. There's so many proteins that we run into a
wall of possibilities. 06:11

companies on this. 06:26

Q Would you agree it's fair to say that he has a financial interest to show that hydrophobic amino acids or peptides don't cross the BBB without the specific receptor that he's trying to develop? 06:27

A I wouldn't be able to comment on that.

Q And in your report, did you consider any potential biases of Mr. Partridge or Dr. Partridge? Apologies.

A No. 06:27

Q Do you expect a protein in a piece of bread to generate the same mixture of tripeptide as AQ?

A It would generate many more than AQ.

Q How about the same mixture?

A They would overlap a lot, and there would be many more of them. 06:28

Q Would they overlap 100 percent?

A One slice of bread versus AQ peptides? I'm not sure. They may or may not.

Q How would you go about determining whether they would generate the same mixture of tripeptides, bread and AQ? 06:28

A So you would take humans; you would feed them bread. You know, breads vary a bunch. So you would feed them a bunch of different breads. And then 06:28

you would measure the appearance of, if you could, of the peptides in the blood, the portal vein, upon absorption to see. 06:28

Q Do you think it's possible to run such a study to make that determination? 06:28

A Yes.

Q And you didn't do that in your preparation for the report; right?

A No, I didn't do that study.

Q Do you expect the protein in a hotdog to generate the same mixture of tripeptides? 06:29

A Yes. A lot more.

Q But not 100 percent overlap; is that right?

A So again, using your arithmetic here, probably not. But then we get into this what does that mean? It means that they're so trivial. Yes. 06:29

Q Do you expect a person taking Prevagen to be taking other proteins that would give rise to the same mixture of tripeptides as Prevagen would?

A Yes. 06:29

Q And all of that intake of proteins, do you think there would be complete overlap of tripeptides?

A Yes.

Q How certain are you of that opinion?

A It cannot not be true at some level. 06:30

06:30 There's so many proteins out there in the diet.

Q How about typical proteins that are consumed in the diet?

A So there's two things. There's so many in the diet. Even when you eat a food, it varies by bite to bite. There's this huge variability. All these amino acids that are found in Prevagen, every single one, are in the foods.

And they're in the foods a thousand times more than Prevagen. So that's how it works.

Q I'm just having a hard time understanding the math given that number of tripeptides possible.

A So I think the confusion is the number of tripeptides possible with the number of tripeptides produced. There's a bit of a difference here. So if we say that -- let's assume there's an obscure one produced. It, by definition, is obscure. That's it by definition. It just doesn't work any other way.

Q So there still is a mathematical possibility that there could be a unique tripeptide attributed to AQ; is that right?

A There's a mathematical possibility, yes.

Q I'd like to introduce Exhibit 12. This will be short, and this is my last set of questions here.

EXHIBIT B

Confidential – Subject to Protective Order

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NORTHERN CALIFORNIA

PHILLIP RACIES, On Behalf of Himself and All Others Similarly Situated, Plaintiff, vs. QUINCY BIOSCIENCE, LLC, Defendant.	Case No. 3:15-cv-00292-HSG **EXPERT REPORT OF RICHARD E. GOODMAN, PH.D.** ***Confidential – Subject to Protective Order***

EXPERT REPORT OF RICHARD E. GOODMAN, PH.D.

I. INTRODUCTION

1. I, Richard E. Goodman, Ph.D., submit this expert report at the request of Quincy Bioscience, LLC. ("Quincy") in the above-captioned litigation.

2. The opinions expressed in this Report are subject to amendment, supplementation or modification based on information made available to the parties in the case, or to respond to or rebut issues, statements and opinions advanced by the plaintiff Phillip Racies ("Racies" or "Plaintiff") or Plaintiff's witnesses.

3. If called upon, I am prepared to testify about my background, qualifications, and experience as well as the issues and opinions described in this Report. Furthermore, I anticipate that I may be asked to provide testimony and to consider and respond to arguments that Plaintiff's expert(s) or fact witnesses may raise at any hearing, in reports, and/or at trial.

A. My Background and Qualifications

4. A copy of my *curriculum vitae* is attached as Exhibit A and includes details of my educational, professional, research and employment credentials.

5. I received a Bachelor of Science degree in Biology from Eastern Washington University in 1977, and a Ph.D. degree in Dairy Science from the Ohio State University in 1990. I conducted post-doctoral training in Immunology at Cornell University between 1990 and 1993.

6. I am currently a Research Professor at University of Nebraska – Lincoln, since August, 2004. I mentor M.S. and Ph.D. students in food science, focusing on food allergy, allergenicity and the safety assessment of genetically engineered organisms.

7. I am the Manager of the AllergenOnline.org database that provides a risk assessment tool for GE (GM) crops and novel food proteins and am currently the Chairman of the WHO/IUIS Allergen Nomenclature Subcommittee. I am a Fellow in the American Academy of Allergy, Asthma and Immunology and member of the European Academy of Allergy and

Clinical Immunology as well as the American Chemical Society and the Institute of Food Technologists.

8. I have published fifty-one peer reviewed scientific journal articles and five book chapters and frequently present scientific presentations on the safety assessment of genetically engineered crops in the US, EU, India, China and other countries, focusing on evaluating potential allergenicity, toxicity, including presentations on stability of proteins in the *in vitro* pepsin digestion assay used to evaluate safety of GE proteins, human serum IgE testing and bioinformatics comparisons to allergens and toxins.

9. From 1980-1985, I worked on the standardization of allergenic extracts for diagnosing allergy. My laboratory also developed a database to evaluate potential risks of celiac disease from proteins derived from wheat-family grasses.

10. I have served as an Associate Editor for the journal of Food and Chemical Toxicology and am an ad hoc reviewer for a number of allergy and toxicology journals.

B. <u>Prior Testimony and Compensation</u>

11. I have not testified in a deposition or trial in the previous four years.

12. I am being compensated at my customary rate of $250/hour for my work on this matter. My compensation does not depend in any way on the outcome of this case.

C. <u>Materials Considered and Preparation</u>

13. The opinions and the statements I make in this Report are based on my knowledge, expertise and professional experience. In addition, I rely on and incorporate by reference the documents and information cited in the Report itself and listed in Exhibit B.

II. <u>OPINIONS</u>

14. I am the principal investigator ("PI") and an author of a report of a 2010 study evaluating the potential allergenicity of apoaequorin (referred to here as "the Allergenicity Study

of 2010"). The report from that study was provided to Quincy. A copy of the study report bears Bates numbers QUI 000811 – 836. I have also conducted a bioinformatics analysis of apoaequorin to assess potential allergenic cross-reactivity. (QUI 000523 – 810). Some of the data from these studies were included in an article by Dr. Daniel L. Moran et al. published in 2014 in the journal Regulatory Toxicology and Pharmacology. (Moran et al. 2014; QUI 000837 – 844).

15. In addition, I was the PI on the studies validating the method used in the Allergenicity Study of 2010. In those studies, we established the time required to reach the limit of 10% residual protein as the time of digestion (Ofori-Anti et al., 2008).

16. I have reviewed the expert report of Dr. Bazinet submitted on behalf of Racies and the transcript of Dr. Bazinet's deposition. During his deposition, Dr. Bazinet admitted he was not a specialist on protein digestion. His opinions on the Allergenicity Study of 2010 showed a lack of understanding of what the study was designed to do, and what it showed.

17. Dr. Bazinet thought the test proved that the protein in Quincy's Prevagen products would be completely digested in the stomach of any individual consuming the products. That is incorrect on two levels. The assay was never intended to predict the *in vivo* digestive fate of dietary proteins, both in terms of the kinetics of protein digestion and the end products of protein digestion in the stomach.

A. The Allergenicity Study of 2010 Does Not Indicate "Complete" Digestion of Apoaequorin Consumed by Humans.

18. It is well known in the protein digestion field that a number of studies have demonstrated that normal physiological conditions in the human stomach are not mimicked by the simple *in vitro* pepsin digestion model, which is used to evaluate stability of purified novel proteins as part of the allergenicity risk assessment for genetically engineered crops. Published

evidence also demonstrates that varying amounts of dietary proteins are absorbed in the intestine and can be detected in blood serum and breast milk from normal consumers.

19. As a Research Professor in the Food Allergy Research and Resource Program within the Department of Food Science and Technology, University of Nebraska-Lincoln, and prior to that as an Allergen Program Manager at Monsanto, I have been involved in a number of studies using protease digestion assays to evaluate potential risks of dietary allergy. Some of the studies I conducted were published in peer-reviewed scientific journals. For example, in addition to the Allergenicity Study of 2010 and the Ofori-Anti et al. (2008) article discussed above, I was a co-author on the pepsin-ring trial study that evaluated the time of digestion of a number of common dietary proteins (Thomas et al., 2004).

20. The Thomas et al. (2004) and Ofori-Anti et al. (2008) studies refined the assay characteristics of the assay originally described by Astwood et al, (1996). The Astwood study evaluated different concentrations of pepsin per mg of test protein, to set a standard for *in vitro* assessment of the risk that a test protein might sensitize consumers. In this type of studies, the assay conditions were not intended to mimic physiological digestion conditions. The pepsin digestion assay was intentionally designed to contain an excess concentration of pepsin at a fixed acidic pH of 1.2 or 2.0 with a limited amount of test protein. A review of published studies using assay conditions based on Astwood et al. (1996) demonstrated that many known dietary allergenic proteins are moderately to fully stable in this *in vitro* digestion assay (Bannon et al., 2002).

21. The study reported by Thomas et al. (2004) was carried out in nine laboratories using a common protocol to test digestion. The protocol called for 10 units of pepsin activity per

mg of protein. All the participating laboratories used the same reagents and studied the same 10 purified proteins. The purpose was to evaluate the robustness and reproducibility of the assay.

22. Test proteins reported in Thomas et al. (2004) included potent dietary allergens, moderate and weak allergens, and non-allergens. Prior to the study, many independent laboratories reported highly variable results in terms of digestion rates, using non-uniform protocols. Many had criticized the study design of Astwood et al. (1996) or suggested modifications to evaluate food processing or denaturation on the impact of stability and possibly allergy. (Besler et al., 2001; Buchanan et al., 1997; del Val et al., 1999; Fu, 2002; Fu et al., 2002; Okunuki et al., 2002; Sen et al 2002; and Tanaka et al 2002).

23. In all laboratories reported by Thomas et al. (2004), the mixture of proteins and pepsin were incubated for similar times from 30 seconds to 60 minutes, before the samples were removed and the pepsin was quenched with bicarbonate buffer and heat to stop digestion. All laboratories tested digestion of each protein at pH 1.2 and also at pH 2. The samples were mixed with reducing Laemmli buffer and separated in SDS-PAGE gels, then stained with Coomassie blue to detect residual protein. Control undigested protein and pepsin were included. The time of disappearance of the primary protein band was estimated by representatives of all laboratories for all results.

24. The focus of the assay was to detect the disappearance of primary protein band on the SDS-PAGE gel. During the study reported by Thomas et al. (2004), any appearance of stable protein fragments was also noted. Stable protein fragments that are detectable with this assay would be peptides beyond a certain size. The peptides smaller than approximately 22 amino acids (~2500 Da) and single amino acids would run at the dye front on an SDS-PAGE gel or

even run off the gel, and would not be detected as a band on the gel by subsequent Coomassie blue stain.

25. The study reported by Thomas et al. (2004) showed good agreement between laboratories. The protocol from that study has since been used as a standard method in many laboratories to help evaluate potential risks that a protein of interest might have a higher probability of sensitizing individuals if introduced into the diet compared to proteins that are rapidly digested in this assay. An absolute time of digestion was not agreed to as a clear limit. Rather the consensus was that proteins digested in less than 5 minutes are unlikely to be significant allergens while those that are stable for more than 20 minutes are more likely to be allergenic.

26. The Study by Ofori-Anti et al. (2008) added additional controls to help standardize the assay. A higher purity of pepsin was used along with a recommendation to test the activity of the pepsin as it is prepared to verify the labeled activity from the manufacturer. In addition, a control was added to calibrate the digestion time of each protein to the time taken to reduce the primary protein band to 10% or less of the starting amount since different proteins stain differently with Coomassie blue (Ofori-Anti et al., 2008). The potential impact of using pepsin at half- or twice- the recommended activity was also evaluated and results demonstrated very limited differences in the time for digestion under the standard conditions.

27. An important variable that is understood is that the pH conditions can dramatically influence digestibility. The pH optimum for pepsin digestion has been reported to be between 1.2 and 2.2, with the specific target protein having some impact on the optimum rate (Schlamowitz and Peterson, 1959; Thomas et al., 2002; Ofori-Anti et al., 2008). Some investigators have suggested using a pH >3.0 as a more physiological average acidity in the

stomach for the risk assessment since gastric pH for infants is greater than 3.0 and rises rapidly following ingestion of a test meal (Bourlieu et al. 2014). However, Bohak (1969) reported the activity of porcine pepsin is only 25% at pH 3. Schlamowitz and Peterson (1959) also reported that the efficacy of pepsin in digesting native versus denatured bovine serum albumin and bovine hemoglobin was reduced markedly at pH 3.5 and above and that the protein and state of denaturation markedly influenced the extent of digestion.

28. Furthermore, Russell et al. (1993) reported results of measuring gastric pH in young and elderly human subjects under fasting and fed conditions. They demonstrated that fasting pH averages around 1.3 (1.1-1.6) in a group of adults of age 65 or older, while the pH rises rapidly to an average close to 5.0 within a few minutes following consumption of a standardized meal of a hamburger with two slices of bread, 2 oz. of potato with garnish, then gradually returns to average fasting pH (~2) over approximately 2.5 hours in young (n=24) and elderly subjects (n=79). Their study demonstrated similar pH profiles for most adult subjects (Russell et al., 1993). The study demonstrates that the acidity of the stomach in most adults is far higher than the ideal, controlled conditions (pH 1.2 or pH 2.0) in the test tube assay used to evaluate the potential allergenicity of apoaequorin (Moran et al., 2014).

29. Most investigators recognized that the assay conditions used in the laboratory were not intended to represent physiological conditions in any individual let alone cover the full range of the variations of conditions that would exist in a population of consumers. The digestion of a purified protein in a simple solution of fixed pH and pepsin would not represent the rate of digestion in complex mixtures of a normal diet. Other components of the diet are known and expected to influence digestion by, for example, creating a physical barrier between an ingested protein and pepsin, altering the protein to pepsin ratio, inhibiting pepsin activity by

buffering the pH, or inhibiting the proteolytic activity of pepsin by the action of amino acid sequences in some proteins and other components associated with some proteins.

30. Picariello et al. (2013) reviewed hundreds of studies reported from 1970-2013 on the correlation between the digestion of food proteins and the proteins' function, allergenic/immunogenic potential, and nutritional properties. They noted that the "static" test-tube digestion assays including the Astwood et al. (1996), Thomas et al. (2004) and Ofori-Anti et al. (2008) models do not predict *in vivo* conditions, because the protein-to-pepsin ratio, pH, and matrix are all important variables between individuals as well as during the time a meal is ingested and passes into the intestine within the individual.

31. To summarize, the conditions for *in vitro* protein digestion in these static pepsin digestion studies are optimized and can help distinguish between proteins that are rapidly digested *in vitro* and do not have a clear history of causing dietary allergy, from those that are found to be relatively stable and have a greater probability of causing food allergic reactions.

32. But even with the optimized conditions, the *in vitro* assays do not require that 100% of the test protein be cleaved. The assays are not designed to show 100% cleavage ("complete" digestion), and they do not show that. The assay standard that we have adopted (Ofori-Anti et al., 2008) uses controls to set the time of "digestion" to the time required to reduce the residual intact protein to 10% or less of the starting amount of the intact protein.

B. The Allergenicity Study of 2010 Does Not Support the Plaintiff's View That Apoaequorin Would Be Completely Digested to Single Amino Acids in Humans.

33. Dr. Bazinet also appears to have thought that apoaequorin would be completely digested to single amino acids. There is no evidence of that, and the Allergenicity Study of 2010 does not support this conjecture by Dr. Bazinet.

34. During his deposition, Dr. Bazinet admitted that the Allergenicity Study of 2010 (he referred to it as the "Quincy study") "didn't measure peptides." (Bazinet Depo. Tr. at 122). He "expect[s] there to be some peptides at some level in there." (*Id.*). Further, he admitted that there is "no" evidence that apoaequorin is entirely digested into single amino acids. (*Id.* at 121).

35. I agree there is no evidence that pepsin digestion of proteins, in general, and apoaequorin in particular, would transform the proteins completely into single amino acids.

36. Pepsin is an endopeptidase, cleaving within the protein and not at all susceptible peptide bonds. The sequence of amino acid types within the target protein or peptide will alter the efficiency of cleavage. Thus, the end-product of pepsin digestion will be a mixture of peptides of varied length, depending on the sequence of the target protein or peptide and the conditions during digestion.

37. The Allergenicity Study of 2010 uses the detection of full-size apoaequorin protein (at about 21-22 kDa) as the readout. Beyond the reduction of the amount of full-size apoaequorin in the test sample, the assay was not designed to ascertain what digestive products were generated.

38. The data from the Allergenicity Study of 2010 do not give us a clear indication of the type or amount of the end products from pepsin digestion of apoaequorin under the assay conditions. However, on the images included in the report of the Allergenicity Study of 2010, there is a faint residual band of protein visible at ~ 21 kDa at time 0.5 minutes, and a smear indicating a mixture of low molecular weight peptides (~ 3-4 kDa, consisting of about 30 amino acid residues or more) for 2 minutes or beyond. As noted in the section above, the smallest peptides and single amino acids would run at the dye front on an SDS-PAGE gel or even run off

the gel, and would not be detected as a band on the gel by subsequent Coomassie blue stain. The results are consistent with the generation of peptides as the digestion product of apoaequorin.

39. While the pepsin digestion assay is not designed to confirm the presence of small peptides, the theoretical protease prediction program, PeptideCutter (ExPASy tools, web/expasy.org/cgi-bin/peptide_cutter/peptidecutter.pl) predicts that at pH 1.3, 30 cleavages of each molecule of apoaequorin are possible, with at least 3 peptides remaining that are 10 amino acid residues or longer, even if proteolysis is 100% efficient. At pH 2, 49 cleavages could occur under ideal conditions, with at least 3 peptides remaining of 10 amino acid residues, even if cutting is 100% efficient. Predicted pepsin cutting sites of apoaequorin generated from the ExPasy Tool website are attached as Exhibit C.

40. Therefore, even in an idealized, theoretical situation, pepsin digestion of apoaequorin does *not* transform the protein into only single amino acids. The predominant end product would be peptides, at least some of which are at considerable length (10-mer or longer).

41. In real life, proteolysis is rarely 100% efficient. Furthermore, the computer predictions often over-predict cutting frequency and I would certainly not expect that every molecule of apoaequorin would be cleaved at all predicted sites in a real-life digestive process. There could also be some molecules that survive cleavage as pH changes or if the protein molecules are protected by matrix.

42. To summarize, there is no evidence from the Allergenicity Study of 2010 or anywhere else that apoaequorin is completely digested to single amino acids by pepsin (or any other digestive enzymes for that matter) in an *in vitro* assay, let alone in the normal physiological conditions in a human body before the protein, or peptides generated from the protein, can be absorbed by the body.

43. At the very least, one would not expect the predominant end product of pepsin digestion of a protein to be single amino acids as opposed to peptides. It is well known that pepsin tends to generate a mixture of peptides from its digestion of a susceptible protein.

44. Therefore, one would expect that ingested apoaequorin, or peptides generated from ingested apoaequorin, would exit the stomach and be subject to absorption in the intestine.

45. Dr. Bazinet never presented any evidence that, in humans, ingested apoaequorin would be completely digested in the intestine upon exiting the stomach *before* absorption of the protein or the peptides derived from it can occur. If Dr. Bazinet is allowed to provide further opinions on apoaequorin digestion in the intestine, I reserve the right to provide further opinions in response.

C. Dr. Bazinet's View on "Dilution" Is Wrong.

46. I also wish to comment on Dr. Bazinet's view of "dilution," which is wrong and not supported by even his own deposition testimony.

47. Dr. Bazinet initially opined that "Because the daily dose of apoaequorin in Prevagen is so low (10 mg) relative to daily dietary protein intakes (about 75,000 mg), any amino acid absorbed as a result of ingesting Prevagen would be trivial compared to those amino acid absorbed from a daily diet." (Banizet Expert Report, at paragraph 17).

48. This opinion did not take into account that apoaequorin may be generating *peptides*, which are then absorbed by the body.

49. As discussed above, during his deposition, Dr. Bazinet admitted that apoaequorin would generate peptides. He then testified that there were "over 50" different amino acid residues in dietary proteins in general, but he could not calculate how many different tripeptides and tetrapeptides 50 amino acid residues could make. (Bazinet Depo. Tr. at 287 – 289).

50. The number of possible peptides is very large. It is possible apoaequorin can generate a peptide that is unique or uncommon among peptides generated by all dietary proteins. Dr. Bazinet has not, and cannot, rule out that possibility.

51. If a peptide (specific amino acid sequence) is unique or uncommon, its effect on the human body would not be "diluted" to triviality by other types of peptides that are different in sequence and structure, and likely to be different in function.

52. Dr. Bazinet does not have a sound basis for his "dilution" opinion once the presence of dietary protein-generated peptides is taken into account.

53. As I discussed above, in real life apoaequorin is expected to generate some peptides consisting of 10 or more amino acid residues, with sequences (order) dictated by the sequence of the protein. The likelihood of a peptide of that size from a different source would, by random chance, have the same amino acid sequence as one of these apoaequorin-generated peptides is very close to zero.

54. It is important to note that a bioactive peptide of 9 amino acids is derived from bovine casein by proteolytic digestion in the gastrointestinal tract and that bioactivity has been demonstrated that would be expected only following absorption of the peptide (Cakir-Kiefer et al. 2011). The peptide showed anxiolytic activity based on behavioral tests following digestion.

55. Other studies have shown specific biological activity *in vivo* from peptides derived from milk proteins (Picariello et al. 2010).

56. Additional studies have demonstrated the presence of dietary proteins, or portions thereof, from peanut (2S albumins) and cow's milk (bovine lactoglobulin, about 18 kDa) in the breast milk of normal (non-allergic) mothers following ingestion of peanut or cow's milk respectively (Bernard et al. 2014; Capitan et al. 2015).

57. Intact bovine lactoglobulin proteins appear to be detected in human breast milk following ingestion of cow's milk (Capitan et al. 2015). The sequence of amino acids in bovine lactoglobulin is unique as there is no human counterpart. Thus the detection of peptides of lactoglobulin by a mass spectroscopy technique following fragmentation of a protein having a mass equivalent to intact bovine lactoglobulin demonstrates that some proteins of nearly 178 amino acids were absorbed following ingestion by humans, circulated in the mother's body and secreted in the milk glands with only minor modification or cleavage.

58. Whether the peanut proteins are taken up fully intact or as long peptide fragments is not clear—the Bernard et al. study used an ELISA assay for the peanut Ara h 6 allergen as the readout. (Bernard et al. 2014, at 889-90). The allergen was detected in human breast milk as soon as 30 minutes after ingestion. (*Id.*). Previous studies have demonstrated that mother's milk can elicit food allergies in young children who are sensitized to similar dietary allergens, which would require the presence in the mother's milk of the allergenic protein or peptide fragments derived from the protein of at least 30 amino acid residues in order to bind two IgE antibodies and cross-link mast cell or basophil receptors.

59. In conclusion, Dr. Bazinet's opinion that apoaequorin will be fully digested to individual amino acids or possibly some very small peptide fragments in the stomach of the consumers is not supported by the robust pepsin digestion assay. Dr. Bazinet's opinion is not reasonable. Furthermore, the demonstration that long peptides or full-length dietary proteins can be absorbed and expressed in human breast milk is certainly counter to the assertion that *all* dietary proteins are completely digested. It is incorrect for Dr. Bazinet to state categorically that dietary proteins or major fragments of the proteins could not be absorbed by consumers in a bioactive form at an amount sufficient to cause a biological effect.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge.

Dated: 9 Nov. 2015

Richard E. Goodman

Richard E. Goodman, Ph.D.

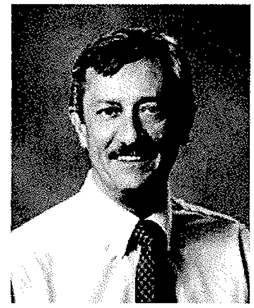
Exhibit A

To the Expert Report of

Dr. Richard E. Goodman, Ph.D.

Curriculum Vitae

CURRICULUM VITAE
Richard E. Goodman, Ph.D.
University of Nebraska
Food Allergy Research and Resource Program
1901 North 21st Street
PO Box 886207
Lincoln, NE 68588-6207
Tel: (402) 472-0452
e-mail: rgoodman2@unl.edu



Citizenship: U.S.A.

PROFESSIONAL EXPERIENCE

1. **Research Professor, Food Science & Technology, Manager of the AllergenOnline.org database University of Nebraska – Lincoln, NE (2004-Present)**
2. **Manager, Allergy Program, Monsanto Company, St. Louis, MO (1997-2004)**
3. **Research Scientist, University of Michigan Medical Center – Pulmonary Division, Ann Arbor, MI (1993-1997)**
4. **Postdoctoral Fellow, Cornell University, School of Veterinary Medicine, Ithaca, NY (1990-1993)**
5. **Product Development Specialist, Hollister-Stier Laboratories, Spokane, WA (1980-1985)**

EDUCATION

1985-1990 Ph.D. The Ohio State University, Columbus, OH. Molecular Biology and Physiology in the Dept. of Dairy Science. Cloned, sequenced and characterized bovine lactoferrin (mammary gland). Grad. Research Asst.

1983-1985 Undergraduate and graduate courses in Business Management. Eastern Washington University, Cheney, WA while working at Hollister-Stier

1977-1980 Graduate studies in Biology. Eastern Washington University, Cheney, WA. Completed course requirements for a Masters Degree and 2 years of research in Botanical Taxonomy. Graduate Teaching Assistant

1973-1977 B.Sc. in Biology, magna cum laude, chemistry minor. Eastern Washington University, Cheney, WA.

GRANTS & AWARDS

2013 Pioneer Hi-Bred International. In vitro serum IgE testing of a stacked-event biotech soybean compared to commercial lines. $218,008; December 2013-December 2014.

2013 Allergen Sequence Database – Bioinformatics Contract renewal (Goodman Co-PI with S. Taylor), renewed, $957,000; January 2013-December 2015

2012	Pioneer Hi-Bred International. Comparison of the relative allergic serum IgE binding between a number of non-GM soybeans and a new GM soybean variety. $226,000; January 2013-June, 2014.
2011	Pioneer Hi-Bred International. Comparison of the relative allergic serum IgE binding between a genetically modified soybean variety and multiple non-soybean varieties.
2011	Bayer CropScience. Comparison of the relative allergic serum IgE binding between a genetically modified soybean variety and multiple non-soybean varieties.
2010	EPA-STAR grant. Co-investigators: Baumert J, Goodman RE, Peterson D. $423,000: Sept 2010-August 2013.
2010	USDA-FAS. Educational activity to develop and coordinate 2 food safety workshops in India ($49,990: 2010: GM food safety, 2011: overall food safety)
2010	USDA-FAS. The Norman E. Borlaug International Agricultural Science and Technology Fellows Program for India. ($39,000. January 2010-December 2011)
2010	USDA-FAS. The Norman E. Borlaug International Agricultural Science and Technology Fellows Program for China. ($20,954. January 2010-December 2010).
2009	BASF Plant Science LLC. Comparison of the relative allergic serum IgE binding between a genetically modified soybean variety and multiple non-soybean varieties. ($45,428. December 2009-March 2010).
2009	Syngenta Crop Science. Specific serum screen of AMY797E a-amylase for IgE binding using serum from Per a 3.01 American cockroach allergic individuals. ($33,122. August 2009-February 2010).
2009	EPA STAR Grant. "Differentiating biologically relevant from irrelevant IgE binding to food antigens for improved risk assessment and diagnostic studies using a humanized rat basophil cell line (RBL 30/25). ($372,340, May 2009-April 2011)
2009	Bill and Melinda Gates Foundation, subaward through Biosafety Resource network for Grand Challenge #9 Projects. Food safety training for international scientists: Allergenicity assessment following Codex 2003 for genetically modified crops. ($112,099, January 2009-December 2009).
2007	Evaluation of the relevance of testing for changes in endogenous allergenicity of GM crops. Bayer CropScience. $22,000. 2007
2007	Allergen Sequence Database – Bioinformatics (Co-PI with S. Taylor), renewed, $617,846, January 2007-December 2009
2006	EPA STAR Grant. "Delineation of appropriate serum IgE testing strategy, protocols and serum donors". ($450,000; Oct. 2006-Sept. 2009).
2004	Monsanto Associate Science Fellow

MEMBERSHIPS IN PROFESSIONAL SOCIETIES

Chairman of the WHO/IUIS Allegen Nomenclature Subcommittee
American Chemical Society (since 2013)

Institute of Food Technologists (since 2008)
European Academy of Allergy and Clinical Immunology (since 2001)
Fellow, American Academy of Allergy, Asthma and Immunology (since 2001)

BIBLIOGRAPHY
Refereed Journals

1. Panda, R, Tetteh, AO, Pramod, SN, Goodman, RE. 2015. Enzymatic hydrolysis does not reduce the biological reactivity of soybean proteins for all allergic subjects. J. Agric. Food Chem. (in press, October, 2015).
2. Siruguri V, Kumar Bharatraj D, Naik Vankudavath R, Rao Mendu VV, Gupta V, **Goodman RE**. 2015. Evaluation of Bar, Barnase and Barstar recombinant proteins expressed in genetically engineered *Brassica juncea* (Indian mustard) for potential risks of food allergy using bioinformatics and literature searches. (In press: Food and Chemical Toxicology, 5 June, 2015.
3. **Goodman RE.** 2014. Biosafety evaluation and regulation of Genetically Modified (GM) crops in the United States. J Huazhong Agricultural University 33(6):85-114 (http://hnxbl.cnjournals.net/hznydxzr/ch/index.aspx English and Chinese available).
4. **Goodman RE**. 2014. GMOs: Are they a regulatory or food safety issue" Cereal Foods World (AACC International). 59(4):164-169.
5. Moran DL, Tetteh AO, **Goodman RE**, Underwood MY. 2014. Safety assessment of the calcium-binding protein, apoaequorin, expressed by Escherichia coli. Regul Toxicol Pharmacol. 69(2):243-249.
6. Ladics GS, Fry J, **Goodman R**, Herouet-Guicheney C, Hoffmann-Sommergruber K, Madsen CB, Penninks A, Pomes A, Roggen EL, Smit J, Wal J-M. Allergic sensitization: screening methods. 2014. Clin Translat Allergy 4:13 DOI:10.1186/2045-7022-4-13.
7. Radauer C, Nandy A, Ferreira F, **Goodman RE**, Larsen JN, Lidholm J, Pomes A, Raulf-Heimsoth M, Rozynek P, Thomas WR, Breiteneder H. 2014. Update of the WHO/IUIS Allergen Nomenclature Database based on analysis of allergen sequences. Allergy E-published January, 2014.
8. **Goodman RE,** Panda R, Ariyarathna H. 2013. Evaluation of endogenous allergens for the safety evaluation of genetically engineered food crops: A review of potential risks, test methods, examples and relevance. J Agri Food Chem 61(35):8317-8332.
9. Zhou C, Sun N, Wang J, Lu J, Tian J, **Goodman RE**, Li N, Che H, Huang K. 2013. Allergenicity assessment of a genetically modified protein-recombinant human lactoferrin. J Allergy Ther S3:002, doi:10 4172/2155-6121.S3-002.
10. Panda R, Ariyarathna H, Amnuaycheewa P, Tetteh A, Pramod SN, Taylor SL, Ballmer-Weber B, **Goodman RE**. 2013. Challenges in testing genetically modified crops for potential increases in endogenous allergen expression for safety. Allergy 68:142-151.
11. Fiocchi A, Burks W, Bahna SL, Boyle RJ, Fuitunen M, Lee BW, Dreborg S, **Goodman R**, Heine RG, Lack G, Cocco R, Haahtela T, Sampson H, Tannock GW,

Osborn DA, Bielory L. 2012. Clinical use of probiotics in pediatric allergy (CUPPA): a World Allergy Organization position paper. WAO Journal 5(11):148-167.

12. Nordlee JA, Panda R, Baumert JL, **Goodman RE**, Taylor SL. 2011. Wild buckwheat is unlikely to pose a risk to buckwheat-allergic individuals. J Food Sci 76(8):T189-T191.
13. Piboonpocanun S, Jirapongsananuruk O, Tipayanon T, Boonchoo S, **Goodman RE**. (2011). Identification of hemocyanin as a novel non-cross-reactive allergen from the giant freshwater shrimp *Macrobrachium rosenbergii*. Mol Nutr Food Res 55(10):1492-1498.
14. **Goodman RE**, Tetteh AO. (2011). Suggested improvements for the allergenicity assessment of genetically modified plants used in foods. Curr Allergy Asthma Rep 11(4):317-324.
15. Vaughan K, Greenbaum J, Kim Y, Vita R, Chung J, Peters B, Broide D, **Goodman R**, Grey H, Sette A. (2010). Towards defining molecular determinants recognized by adaptive immunity in allergic disease: an inventory of available data. J Allergy (Cairo). [E published]
16. Panda R, Taylor SL, **Goodman RE**. (2010). Development of a sandwich enzyme linked immunosorbent assays (ELISA) for detection of buckwheat residues in food. J Food Sci. 75(6):T110-T117.
17. Ladics GS, Knippels LMJ, Penninks AH, Bannon GA, **Goodman RE**, Herouet-Guicheney. (2010). Review of animal models designed to predict the potential allergenicity of novel proteins in genetically modified crops. Reg Toxicol Pharma 56:212-224.
18. **Goodman, RE**. (2008). Performing IgE serum testing due to bioinformatics matches in the allergenicity assessment of GM crops. Food Chem Toxicol 46:S24-S34.
19. Ofori-Anti AO, Ariyarathna H, Chen L, Lee HL, Pramod SN, **Goodman RE**. (2008). Establishing objective detection limits for the pepsin digestion assay used in the assessment of genetically modified foods. Reg Tox Pharmacol 52:94-103.
20. **Goodman RE**, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL, van Ree R. (2008). Allergenicity assessment of genetically modified crops – what makes sense? Nat Biotech 26(1):73-81.
21. Hoff M, Son DY, Gubesch M, Ahn K, Lee SI, Vieths S, **Goodman RE**, Ballmer-Weber BK, Bannon GA. (2007). Serum testing of genetically modified soybeans with special emphasis on potential allergenicity of the heterologous protein CP4EPSPS. Mol Nutr Food Res July 2007 51:946-955.
22. Peeters KABM, Nordlee JA, Penninks AH, Chen L, **Goodman RE**, Bruijnzeel-Koomen CAFM, Hefle SL, Taylor SL, Knulst AC. (2007). Lupine allergy: not simply cross-reactivity with peanut or soy. J Allergy Clin Immunol 120(3):647-653.
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26. Chen L, Lucas JS, Hourihane JOB, Lindemann J, Taylor SL, **Goodman RE**. (2006). Evaluation of IgE binding to proteins of hardy (Actinidia arguta), gold (Actinidia chinensis) and green (Actinidia deliciosa) kiwifruits and processed hardy kiwifruit concentrate, using sera of individuals with food allergies to green kiwifruit. Food and Chem Toxicol. 44(7):1100-1107.
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32. **Goodman RE**, Silvanovich A, Hileman RE, Bannon GA, Rice EA, Astwood JD. 2002. Bioinformatic methods for identifying known or potential allergens in the safety assessment of genetically modified crops. Comments on Toxicology 8:251-269.
33. Bannon GA, **Goodman RE**, Leach JN, Rice E, Fuchs RL, Astwood JD. 2002. Digestive stability in the context of assessing the potential allergenicity of food proteins. Comments on Toxicology 8:271-285.
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35. Chan SY, **Goodman RE**, Szmuszkovicz JR, Roessler B, Eichwald EJ, Bishop DK. 2000. DNA-liposome versus adenoviral mediated gene transfer of transforming growth factor beta 1 in vascularized cardiac allografts: differential sensitivity of CD4+ and CD8+ T cells to transforming growth factor beta 1. Transplantation 70(9): 1292-1301.
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induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 279(3): L487-495.

37. Christensen PJ, **Goodman RE**, Pastoriza L, Moore B, Toews GB. Induction of lung fibrosis in the mouse by intratracheal instillation of fluorescein isothiocyanate is not T-cell-dependent. Am J Pathol (1999) 155(5):1773-9.
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49. Schanbacher FL, **Goodman RE**, Talhouk RS. Bovine mammary lactoferrin: implications from messenger ribonucleic acid (mRNA) sequence and regulation contrary to other milk proteins. J Dairy Sci (1993) 76 (12): 3812-31.
50. **Goodman RE**, Oblak J, Bell RG. Synthesis and characterization of rat interleukin-10 (IL-10) cDNA clones from the RNA of cultured OX8-- OX22-- thoracic duct T cells. Biochem Biophys Res. Comm. (1992) 189:1-7.

51. **Goodman RE**, Schanbacher FL. Bovine lactoferrin mRNA: Sequence, analysis and expression in the mammary gland. Biochem Biophys Res Comm (1991) 180:75-84.

Book Chapters

1. **Goodman, RE**, Ofori-Anti AO. Assessing the potential allergenicity of Genetically Modified (GM) Cowpea following CODEX Alimentarius Guidelines (2003), pp 162-177. In: Innovative research along the cowpea value chain. 2012 Proceedings of the 5th International Cowpea Symposium, Saly, Senegal. 27 September-1 October 2010, edited by O. Boukar, O. Coulibaly, C.A. Fatokun, K. Lopez and M. Tamo, IITA, Nigeria. 432 pp.
2. **Goodman, RE.** Clinical food allergy and allergens. In: Food Safety, Quality Assurance and Global Trade. SP Singh, J Funk, SC Tripathi and N Joshi *eds.* International Book Distributing, Co. Lucknow, India; 2009:189-199.
3. **Goodman, RE.** Genetically modified crop safety (food/feed): human and animal health. In: Food Safety, Quality Assurance and Global Trade. SP Singh, J Funk, SC Tripathi and N Joshi *eds.* International Book Distributing, Co. Lucknow, India; 2009:49-56.
4. **Goodman, RE**, Wise J. Predicting the allergenicity of novel proteins in genetically modified organisms. Food Allergy, SJ Maleki, AW Burks, RM Helm *eds.* American Society for Microbiology Press, Washington, DC; 2006:219-247.
5. Hamilton KA, **Goodman RE**, Fuchs RL. Chapter 16. Safety assessment of insect-protected cotton. Genetically Modified Crops, J Thomas R. Fuchs *eds.* Elsevier, Inc.; 2002, 3rd edition. pp435-465.

Students Mentored:

1. Afua Ofori-Anti, PhD, completed August 2010.
2. Harsha Ariyarathna, MSc, completed June 2009.
3. Rakhi Panda, MSc, completed August 2009.
4. Rakhi Panda, PhD, completed December, 2012.
5. Plaimein Amnuaycheewa, PhD, completed August, 2014.
6. Nathan Marsteller, PhD, completed December, 2014.
7. Fulei Luan, PhD, expected May, 2015.
8. Kwami Andho-Kumi, PhD, expected December, 2015.
9. Mei Lu, PhD, expected December, 2015.
10. Yuan Jin, PhD, expected December, 2016

Exhibit B

To the Expert Report of

Richard E. Goodman, Ph.D.

Additional Materials Considered

MATERIALS CONSIDERED

Journal Articles:

Astwood, J.D. et al., Stability of food allergens to digestion in vitro. Nat. Biotechnol. 1996. 14:1269-1273.

Bannon, F.A. et al., Digestive stability in the context of assessing the potential allergenicity of food proteins. Comments Toxicol. 2002. 8:271-285.

Bernard, H. et al., Peanut allergens are rapidly transferred in human breast milk and can prevent sensitization in mice. Allergy. 2014. 69:888-897;

Besler, M. et al., Stability of food allergens an dallergenicity of processed foods. J. Chromatogr. B. Biomed. Sci. Appl. 2001. 756(1-2): 207-228.

Bohak, Z. et al., Purification and Characterization of Chicken Pepsinogen and Chicken Pepsin. J Biol Chem. September 10, 1969. 244(17):4638-48.

Bourlieu, C., et al., Specificity of infant digestive conditions: Some clues for developing relevant in vitro models. Crit. Rev. Food Sci. Nutr. 54(11): 1427-1457.

Buchanan, B.B. et al., Thioredoxin-linked mitigation of allergic responses to wheat. Proc. Natl. Acad. Sci. USA, 1997. 94:5372-5377.

Cakir-Kiefer, C. et al., In vitro digestibility of alpha-casozepine, a benzodiazepine-like peptide from bovine casein, and biological activity of its main proteolytic fragment. J. Agric. Food Chem. 2011, 59(9): 4464-4472.

Capitan, F. et al., β-Lactoglobulin detected in human milk forms noncovalent complexes with maltooligosaccharides as revealed by chip-nanoelectrospray high-resolution tandem mass spectrometry. Amino Acids. November 2015. 47(11): 2399-407.

del Val, G. et al., Thioredoxin treatment increases digestibility and lowers allergenicity of milk. J. Allergy Clin. Immunol. 1999. 103:690-697.

Fu, T.J. et al., Digestion stability as a criterion for protein allergenicity assessment. Ann. N.Y. Acad. Sci. 2002. 964:99-110.

Fu, T.J. et al., Digestibility of food allergens and non-allergenic proteins in simulated gastric and intestinal fluids—a comparative study. J. Agric. Food Chem. 2002. 50:7154-7160.

Kenna, J.G. et al., Digestibility of proteins in simulated gastric fluid. The Toxicoloist. 2000. 54(1). Abstract 666.

Moran, D.L. et al., Safety assessment of the calcium-binding protein, apoaequorin, expressed by Escherichia coli. Reg. Toxicol. Pharmacol. 2014. 69:243-249. (QUI 0000837-44).

Ofori-Anti, A.O. et al., Establishing objective detection limits for the pepsin digestion assay used in the assessment of genetically modified foods. Regul. Toxicol. Pharmacol. 2008. 52:94-103.

Okunuki, H. et al., Increased digestibility of two products in genetically modified food (CP4-EPSPS and Cry1Ab) after preheating. J. Food Hyg. Soc. Japan. 2002. 43:68-73.

Picariello, G. et al., Peptides surviving the simulated gastrointestinal digestion of milk proteins: biological and toxicological implications. J. Chromatogr B Analyt. Technol. Biomed. Life Sci. 2010. 878(3-4):295-308.

Picariello, G. et al., Protein digestomics: Integrative platforms to study food-protein digestion and derived functional and active peptides. Trends Anal. Chem. 2013. 52:120-134.

Russell, T.L. et al., Upper Gastrointestinal pH in Seventy-Nine Healthy, Elderly, North American Men and Women. Pharm. Res. 1993. 10(2): 187-96.

Schlamowitz, M. and Peterson, L.U. Studies on the Optimum pH for the Action of Pepsin on "Native" and Denatured Bovine Serum Albumin and Denatured Bovine Serum Albumin and Bovine Hemoglobin. The Journal of Biological Chemistry. December 1959. 234(12): 3137-45.

Sen, M. M., Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. J. Immunol. 2002. 169:882-887.

Tanaka, K., et al., Pepsin-resistant 16 kDa Buckwheat protein is associated with immediate hypersensitivity reactions in patients with Buckwheat allergy. Int. Arch. Allergy Immunol. 2002. 129:49-56.

Thomas, K., et al., A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Regul. Toxicol. Pharmacol. 2004. 39:87-98.

Other documents:

QUI 0000523-810 (Report on bioinformatics analysis)

QUI 0000811-36 (Report on allergenicity study)

Expert Report of Richard P. Bazinet, Ph.D.

Transcript of the deposition of Dr. Richard Bazinet, Oct. 16, 2015.

Exhibit C

To the Expert Report of

Dr. Richard E. Goodman, Ph.D.

PeptideCutter

The sequence to investigate:

```
        10         20         30         40         50         60
MTSKQYSVKL TSDFDNPRWI GRHKHMFNFL DVNHNGKISL DEMVYKASDI VINNLGATPE

        70         80         90        100        110        120
QAKRHKDAVE AFFGGAGMKY GVETDWPAYI EGWKKLATDE LEKYAKNEPT LIRIWGDALF

       130        140        150        160        170        180
DIVDKDQNGA ITLDEWKAYT KAAGIIQSSE DCEETFRVCD IDESGQLDVD EMTRQHLGFW

       190
YTMDPACEKL YGGAVP
```

The sequence is 196 amino acids long.

Available enzymes

The enzyme(s) that you have chosen:

- Pepsin (pH1.3)
- Pepsin (pH>2)

You have chosen to display all possible cleaving enzymes.

These enzymes cleave the sequence:

Name of enzyme	No. of cleavages	Positions of cleavage sites
Pepsin (pH1.3)	30	9 10 13 14 28 29 30 40 54 55 71 72 73 95 100 101 111 118 119 120 132 133 155 156 166 167 177 179 189 190
Pepsin (pH>2)	48	5 9 10 13 14 19 28 29 30 40 44 45 54 55 71 72 73 79 80 86 89 92 93 95 100 101 103 104 111 114 118 119 120 132 133 135 136 138 155 156 166 167 177 179 180 181 189 190

These are the cleavage sites of the chosen enzymes and chemicals mapped onto the entered protein sequence:

- You have chosen a block size of **60** for the map.
- Please note that the cleavage occurs at the **right side** (C-terminal direction) of the marked amino acid.
- You have the possibility to display the results of a single enzyme by **mouseclicking** on the respective enzyme name in the map.

```
         Pn1.3_Pn2
        Pn1.3_Pn2|         Pn1.3_Pn2                 Pn1.3_Pn2
     Pn1.3_Pn2   ||       Pn1.3_Pn2|                Pn1.3_Pn2|
    Pn1.3_Pn2|   ||      Pn1.3_Pn2||            Pn2         ||
      Pn2   ||   ||   Pn2         ||| Pn1.3_Pn2 Pn2|        ||
```

```
         |    ||   ||      |         |||          |    ||          ||
     MTSKQYSVKLTSDFDNPRWIGRHKHMFNFLDVNHNGKISLDEMVYKASDIVINNLGATPE
  1  ---------+---------+---------+---------+---------+---------+   60


                                                            Pn1.3_Pn2
                                                           Pn1.3_Pn2|
                                                          Pn1.3_Pn2||
                                                            Pn2    |||
                                                   Pn1.3_Pn2   |   |||
                                                  Pn2       |  |   |||
                                                 Pn2|       |  |   |||
                                         Pn1.3_Pn2 ||       |  |   |||
                                        Pn1.3_Pn2| ||       |  |   |||
                                   Pn1.3_Pn2    || ||       |  |   |||
                                       Pn2 |    || ||       |  |   |||
        Pn1.3_Pn2                     Pn2| |    || ||       |  |   |||
       Pn1.3_Pn2|     Pn2         Pn2  || |    || ||       |  |   |||
      Pn1.3_Pn2||    Pn2|    Pn2   |   || |    || ||       |  |   |||

               |||      ||      |  |   || |    || ||       |  |   |||
     QAKRHKDAVEAFFGGAGMKYGVETDWPAYIEGWKKLATDELEKYAKNEPTLIRIWGDALF
 61  ---------+---------+---------+---------+---------+---------+   120


                     Pn2
                   Pn2 |
                  Pn2| |                                                Pn2
         Pn1.3_Pn2 || |           Pn1.3_Pn2  Pn1.3_Pn2   Pn1.3_Pn2|
        Pn1.3_Pn2| || |          Pn1.3_Pn2| Pn1.3_Pn2| Pn1.3_Pn2 ||

                ||  || |                ||         ||         |  ||
     DIVDKDQNGAITLDEWKAYTKAAGIIQSSEDCEETFRVCDIDESGQLDVDEMTRQHLGFW
121  ---------+---------+---------+---------+---------+---------+   180


      Pn1.3_Pn2
     Pn1.3_Pn2|
   Pn2       ||

     |       ||
     YTMDPACEKLYGGAVP
181  ---------+------   196
```

EXHIBIT C

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NORTHERN CALIFORNIA

PHILLIP RACIES, On Behalf of Himself and All Others Similarly Situated, Plaintiff, vs. QUINCY BIOSCIENCE, LLC, Defendant.	Case No. 3:15-cv-00292-HSG **EXPERT REPORT OF MICHAEL A. PEZZONE, M.D., PH.D.**

EXPERT REPORT OF MICHAEL A. PEZZONE, M.D., PH.D.

I. INTRODUCTION

1. I, Michael Pezzone, submit this expert report at the request of Quincy Bioscience, LLC. ("Quincy") in the above-captioned litigation.

2. The opinions expressed in this Report are subject to amendment, supplementation or modification based on information made available to the parties in the case, or to respond to or rebut issues, statements and opinions advanced by the plaintiff Phillip Racies ("Plaintiff") or his witnesses.

3. If called upon, I am prepared to testify about my background, qualifications, and experience as well as about the issues and opinions described in this Report. Furthermore, I anticipate that I may be asked to provide testimony and to consider and respond to arguments that Plaintiff's expert(s) or fact witnesses may raise at any hearing, in reports, and/or at trial.

A. My Background and Qualifications

4. A copy of my recent *curriculum vitae* is attached as Exhibit A and includes details of my educational, professional, research and employment credentials.

5. I received a Bachelor of Science degree in Chemistry and Biochemistry from Cornell University in 1987. I studied the mechanisms of carbohydrate hydrolysis, which was the topic of my Honors thesis. My other pre-doctoral research projects included the study of autoimmune disorders including an autoimmune pathogenesis of schizophrenia and the incorporation of cardiac calcium channels into lipid bilayers to study their properties.

6. I received dual M.D. and Ph.D. degrees from the University of Pittsburgh School of Medicine in 1994. My doctoral thesis discussed the central nervous system pathways that are activated by acute and conditioned stress and their role in the

suppression of the peripheral immune system via the autonomic nervous system and the hypothalamic-pituitary-adrenal axis.

7. I am currently an Adjunct Associate Professor of Pharmacology & Chemical Biology at the University of Pittsburgh School of Medicine. I also have an appointment at the McGowan Institute for Regenerative Medicine.

8. I have had extensive funding by the National Institute of Health (NIH) and other agencies to study stress effects on the immune system, neurogenic (nerve-mediated) inflammation in the bowel, and a neurogenic pathogenesis of bowel-bladder cross-sensitization as it applies to the overlap of irritable bowel syndrome and interstitial cystitis. My studies specifically focused on the role of mast cells and their effects on afferent (sensory) pain nerves including their sensitization and the associated mucosal permeability changes in both the bowel and bladder which can lead to a further disease cascade.

9. I have been practicing medicine for more than 21 years, and am currently certified by the American Board of Internal Medicine, the American Board of Gastroenterology, and the National Board of Medical Examiners. I am a Fellow (Life Member) of the American Gastroenterological Association and a member of the American College of Gastroenterology and the American Association for the Study of Liver Diseases.

B. <u>Prior Testimony and Compensation</u>

10. During the past four years, I have provided expert testimony at trial and/or a deposition in *Marks v. Feng (*2013), Case Number CV 12 789848, Court of Common Pleas of Cuyahoga County, Cleveland, OH.

11. I am being compensated at my customary rate of $550/hour for my work on this matter. My compensation does not depend in any way on the outcome of this case.

C. Materials Considered and Preparation

12. The opinions and the statements I make in this Report are based on my knowledge, expertise and professional experience. In addition, I rely on and incorporate by reference the documents and information cited in the Report itself and listed in Exhibit B attached to this Report.

II. OPINIONS

13. I was asked to opine on whether proteins, including apoaequorin, can be absorbed through the gastrointestinal tract in humans, and whether animal studies addressing this issue are applicable to humans.

14. In my opinion, the answer to both of these questions is "yes."

A. Proteins Can Be Absorbed through the Human Gastrointestinal Tract.

15. It has been shown conclusively that macromolecule uptake in the human small intestine can occur under physiological conditions and in antigenic and biologically active quantities (Lorkowski Review).[1]

16. In healthy persons, the absorption of small amounts of dietary proteins[2] from the gastrointestinal tract has been observed with no deleterious effects (Paganelli, Husby 1985). The development of serum antibodies to dietary antigens, a reflection of

[1] Please refer to Exhibit B attached to this Report ("Additional Materials Considered") for full citations of the references discussed in the Report.

[2] Protein is a large peptide chemically. A mechanism of absorption for a protein is also applicable to large peptides. Therefore, the term "protein" as used in this report includes large peptides that may be derived from an ingested protein.

protein absorption, is thought to be a normal physiologic response after the ingestion of food and may play a role in oral tolerance (Husby 2000).

17. In humans, the absorption of ingested β-lactoglobulin (Jakobsson), ovalbumin (Husby 1986, Dannaeus), bovine serum albumin (Paganelli), and horseradish peroxidase (Heyman) have all been demonstrated.

18. Similarly, absorption of horseradish peroxidase (Walker), bovine serum albumin (Worthington), ovalbumin (Poriadkov), endotoxin (Ravin), lysozyme (Yokooji), azo dyes (Barnett), latex particles (Sanders), and even viable bacteria (Schatten) has been reported in animals.

19. The absorption of ingested proteins in humans and animals shows that proteins are not necessarily digested to completion after ingestion—a significant portion of an ingested protein could survive digestion and be absorbed across the gastrointestinal tract.

20. In the context of a protein-containing product ingested by many consumers, the variability among a human population with respect to protein absorption should be taken into account. In humans, stress, surgical trauma (Rhodes), diseases such as celiac disease, alcoholic liver disease (Parlesak), NSAID[3] use (Yokooji), and increasing age can all lead to increased intestinal permeability including opening of tight junctions and may further accentuate this process. Recent advances in the measurement of intestinal permeability will shed further light on many of the above disease processes including such common conditions as irritable bowel syndrome.

[3] Common NSAIDs include, for example, aspirin and ibuprofen.

21. Proteins that are more acid- and pepsin-stable are more readily absorbed in the small intestine after ingestion, and, in terms of allergenicity, more active. Achlorhydric states including those induced by proton pump inhibitors (e.g. omeprazole/Prilosec® and esomeprazole/Nexium®), which are used widely, may facilitate the protein absorption process and may have substantial implications. Specific mechanisms of macromolecule (including proteins) absorption include endocytosis, paracellular absorption, and M cell transport (Lorkowski).

22. I have reviewed the Court's Order dismissing Plaintiff's claims based on "lack of substantiation." I understand that Plaintiff must come forth with evidence and prove that apoaequorin is completely and fully digested after a consumer takes Quincy's product Prevagen®, and that the digestion product of apoaequorin would be absorbed in "trivial" amounts.

23. I have reviewed the deposition transcript of Plaintiff's expert Dr. Richard Bazinet. I do not believe Dr. Bazinet has provided any evidence to prove these points.

24. Absent any evidence to the contrary, the discussion above regarding the existence, significance and mechanisms of protein absorption in humans is applicable to apoaequorin. One cannot rule out the possibility that apoaequorin, or a peptide derived from apoaequorin, can utilize one or more of the known mechanisms of absorption in the small intestine or elsewhere, across the gastrointestinal tract, in non-trivial amounts.

B. Animal Models Have Applicability to Humans.

25. Researchers have been generally aware that animal research models are not always completely applicable to human disease states. However, given the fundamental role of the gastrointestinal tract across animal species in the absorption of nutrients, sampling of antigenic stimuli, immune tolerance, and the passage of waste, etc.,

one would expect that animal studies of macromolecule absorption would reflect the human condition, as the studies discussed above and many other studies indicate.

26. In fact, animal models have been widely used in the study of protein absorption across the gastrointestinal tract, and there is no indication that researchers would stop using animal models in this field of study to obtain information that is applicable to humans.

27. The rat model is often used initially in this field of study. (*See*, *e.g.*, Walker, Worthington, Yokooji). Subsequently, after determination of the mechanism of absorption and bioavailability in small animals such as the rat, larger animals such as dogs, pigs, and monkeys are used to assess absorption from oral formulations (Kararli Review).

28. Dogs have been used extensively prior to the introduction of drugs into humans. Dogs and humans have similar stomach morphology and emptying characteristics, similar overall GI tract dimensions, and comparable drug availability (Kararli). In addition, dogs also have M cells, which contain multiple vesicles used in the transport of luminal peptides, proteins, and antigens (Kararli). As such and paralleling human studies (Husby 1986, Dannaeus), studies in dogs that measured absorption and immune responses to oral ovalbumin, a 45 kDa protein, have shown systemic antibody responses which were detectable within 15 days (Poriadkov).

29. Because protein absorption occurs through such basic, conserved cellular processes (endocytosis, pinocytosis, paracellular absorption, etc.), one would expect that the applicability of animal models to the human condition would be quite high.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge.

Dated: November 9, 2015

Michael A. Pezzone, M.D., Ph.D.

Exhibit A

To the Expert Report of

Dr. Michael A. Pezzone, M.D., Ph.D.

Curriculum Vitae

BIOGRAPHICAL

Name: Michael A. Pezzone, M.D., Ph.D., A.G.A.F.

Business Address: Manifold Professional Bldg. #3
86 Wellness Way
Washington, PA 15301

E-Mail: pezzone@pitt.edu
pezzonem@gmail.com
Website: DrPezzone.com

Department of Pharmacology & Chemical Biology
13th Floor Biomedical Science Tower, Pittsburgh, PA 15261

Business Phone: (724) 503-4637 **Fax:** (724) 503-4429

EDUCATION and TRAINING

UNDERGRADUATE:

Dates Attended	*Name and Location of Institution*	*Degree Received and Year*	*Major Subject*
1983-87	Cornell University Ithaca, NY	B.A. 1987 Magna Cum Laude	Chemistry Biology

Honor's Thesis: "Structure-Activity Relationships in Glycosidase Inhibitors."
Bruce Ganem, Ph.D., Professor and Chairman of Chemistry, Advisor

PRE-DOCTORAL:

Dates Attended	*Name and Location of Institution*	*Name of Program Director and Discipline*
Summer 1987	University of Pittsburgh School of Medicine and Western Psychiatric Institute & Clinic	David Kupfer, M.D. Rohan Ganguli, M.D. Robert Kelly, Ph.D.

Mellon Pre-Doctoral Fellow in Psychiatry. Project: "An Autoimmune Basis for Schizophrenia." Departments of Psychiatry and Immunopathology.

GRADUATE:

Dates Attended	*Name and Location of Institution*	*Degree Received and Year*	*Major Advisor and Discipline*
1987-94	University of Pittsburgh School of Medicine	M.D., Ph.D. 1994	Pathology Neuroscience

Dissertation: "Characterization of the Pathways Mediating Stress-Induced Immune Alterations in the Rat." Bruce Rabin, M.D., Ph.D., Professor of Pathology and Psychiatry, Advisor.

POSTGRADUATE:

Dates Attended		*Name and Location of Institution*	*Name of Program Director and Discipline*
1994-95	Internship	University of Pittsburgh Medical Center	Frank Kroboth, M.D. Internal Medicine
1995-97	Residency	University of Pittsburgh Medical Center	Frank Kroboth, M.D. Internal Medicine
1997-00	Fellowship	University of Pittsburgh Medical Center	Arnold Wald, M.D. Gastroenterology & Hepatology

Research Mentor: William C. de Groat, Ph.D., Professor of Pharmacology
Clinical Mentors: Adam Slivka, M.D., Ph.D., Chief of Endoscopy
Arnold Wald, M.D., Director of Motility Lab

APPOINTMENTS and POSITIONS

ACADEMIC:

Years Inclusive	*Name and Location of Institution*	*Rank/Title*
11/09-Present	University of Pittsburgh School of Medicine	Adjunct Associate Professor of Pharmacology & Chemical Biology
2005-Present	McGowan Center for Regenerative Medicine, University of Pittsburgh and UPMC	Secondary Appointment
1/13-Present	Duquesne University Pittsburgh, PA	Clinical Preceptor Department of Physician Assistant Studies
5/08-10/31/09	University of Pittsburgh School of Medicine	Associate Professor Medicine (Tenure stream)
5/08-10/31/09	University of Pittsburgh School of Medicine	Associate Professor Pharmacology & Chemical Biology (Secondary Appointment)

10/01-4/08	University of Pittsburgh School of Medicine	Assistant Professor Medicine (Tenure stream)
10/01-4/08	University of Pittsburgh School of Medicine	Assistant Professor Pharmacology & Chemical Biology (Secondary Appointment)
2/99-9/01	University of Pittsburgh School of Medicine	Instructor Medicine
9/99-9/01	University of Pittsburgh School of Medicine	Instructor Pharmacology (Secondary Appointment)

NON-ACADEMIC:

Years Inclusive	***Name and Location of Institution***	***Rank/Title***
2014-Present	East Liverpool City Hospital East Liverpool, OH	Staff Physician
2009-Present	Washington Hospital Washington, PA	Staff Physician
2014-Present	UPMC-St. Margaret Fox Chapel, PA	Staff Physician
2011-Present	St. Clair Hospital Pittsburgh, PA	Staff Physician
1997-99, 2009-present	Mercy Hospital Pittsburgh, PA	Staff Physician
2010-2013	UPMC-Passavant	Staff Physician
2000-Present	UPMC-Presbyterian Shadyside UPMC-Montefiore, Magee, Southside UPMC South Surgical Center	Staff Physician
1996-99	St. Clair Hospital Pittsburgh, PA	House Physician

CERTIFICATION and LICENSURE

SPECIALTY CERTIFICATION:

Certifying Board	*Year*
Gastroenterology	2000-2020
Internal Medicine	1997-2017

MEDICAL or OTHER PROFESSIONAL LICENSURE:

Licensing Board/State	*Year*
Pennsylvania	1996-Present
Ohio	2014-Present

CURRENT CLINICAL PRACTICE

Includes general gastroenterology, hepatology, pancreaticobiliary, motility, functional bowel disorders, inflammatory bowel disease, and the treatment of liver diseases. Trained in motility by Dr. Arnold Wald.

Clinical trials for IBS, Constipation, and Diarrhea—see below.

Active collaboration with Dr. Steven Badylak at the McGowan Institute for Regenerative medicine investigating the cytokine response to extracellular matrix (ECM) and determination of the phenotype of immune cells in fixed specimens from patients with ulcerative colitis

Procedural Skills: (and current volumes)
Therapeutic Endoscopy: Endoscopy (~600/yr.), Colonoscopy (~1600/yr.), Therapeutic ERCP (~30/yr.), Video Pill Enteroscopy (~40/yr.); Esophageal and Rectal Manometry, pH studies; Esophageal, Enteral, Pancreatic/Biliary and Colonic Stents; EMR (Provation; Epic; Sunrise; NextGen; E-Clinical Works).

MEMBERSHIPS in PROFESSIONAL and SCIENTIFIC SOCIETIES

Organization	*Year*
•American Association for the Study of Liver Diseases	2012-present
•American College of Gastroenterology	1998-present
•American Society for Gastrointestinal Endoscopy	1998-2001
•American College of Physicians	1996-2000
•American Gastroenterological Association	1995-present
•American Association for the Advancement of Science	1992-present
•The Society for Neuroscience	1991-present
•The Psychoneuroimmunology Research Society-Charter member	1993-2000
•Brain, Behavior and Immunity Center, The University of Pittsburgh and Carnegie Mellon University-Charter member	1990-present

HONORS

Title of Award	*Year*
•Certificate of Achievement, Enterprise Development, "From Bench to Bedside: What Every Scientist Needs to Know"	2015
•Fellow, American Gastroenterological Association	2010-Present
•Castle Connolly Top Doctor	2008-Present
•Pittsburgh Magazine's "Top Doctor" in Gastroenterology	2008-2012
•Patients' Choice Recognition Award	2010
•PURE HOPE 4th Annual Women's Pelvic Health Conference--Keynote Speaker, Houston, TX	2009
•Pittsburgh Magazine's "Top Doctor" in Gastroenterology (1 of 3 awardees)	2008
•Nominated to "America's Top Doctors"	2008
•University of Pittsburgh Medical Center 20-year Service Award	2008
•Recognition for Service Excellence "Above and Beyond"	2007
•Audrey Love Charitable Foundation Award for Research in IBS	2007
•International Pelvic Pain Society Annual Meeting—Best Basic Research Presentation	2006
•Research Excellence in GI and Liver (REGAL) Award—Lower GI Research	2005
•International Foundation for Functional Gastrointestinal Disorders (IFFGD) Junior Investigator—Basic Science Award	2005
•Research Insight into Interstitial Cystitis (Abstract Award Winner)	2003
•AGA Distinguished Abstract (Plenary Session Oral Presentation)	2002
•AGA Academic Skills Workshop Attendee	2002
•The Samuel and Emma Winters Foundation Award for Biomedical Research	2001
•ASGE Fifth Annual Young Investigators' Conference in Digestive Diseases, *Best Poster Presentation*	2000
•Medical Scientist Training Program M.D./Ph.D. Scholarship, *Mellon Foundation*	1987-94
•Pittsburgh Neuroscience Society, *Graduate Student Research Prize, Best Paper*	1992
•Mellon Pre-Doctoral Fellowship in Psychiatry, *Western Psychiatric Institute &*	1987

Clinic, University of Pittsburgh, School of Medicine

•Magna Cum Laude, Honors in Chemistry, *Cornell University*	1987
•Arts & Sciences Dean's Scholarship, *Cornell University*	1985-87
•Valedictorian, *New Castle Senior High School, New Castle, PA*	1983
•Presidential Classroom for Young Americans Awardee, *New Castle, PA*	1983

PUBLICATIONS

Refereed Articles

1. Bernotas, R.C., **Pezzone, M.A.**, & Ganem, B., Synthesis of (+)-1,5-dideoxy-1,5-imino-D-galactitol, a potent alpha-D-galactosidase inhibitor, *Carbohydrate Research*, 167 (1987) 305-311.

2. **Pezzone, M.A.**, Rush, K.A., Kusnecov, A.W., Wood, P.G., and Rabin, B.S., Corticosterone-independent alteration of lymphocyte function by amphetamine, *Brain, Behavior & Immunity*, 6 (1992) 293-299.

3. **Pezzone, M.A.**, Lee, W.-S., Hoffman, G.E., and Rabin, B.S., Induction of c-Fos immunoreactivity in the rat forebrain by conditioned and unconditioned aversive stimuli, *Brain Research*, 597 (1992) 41-50. **(Classic Paper)**

4. **Pezzone, M.A.**, Lee, W.-S., Hoffman, G.E., Pezzone, K.M., and Rabin, B.S. Activation of brainstem catecholaminergic neurons by conditioned and unconditioned aversive stimuli as revealed by c-Fos immunoreactivity, *Brain Research*, 608 (1993) 310-318. **(Classic Paper)**

5. **Pezzone, M.A.**, Dohanics, J., and Rabin, B.S. Effects of footshock stress upon spleen and peripheral blood lymphocyte mitogenic responses in paraventricular nucleus (PVN) lesioned rats, *Journal of Neuroimmunology*, 53 (1994) 39-46.

6. Shanks, N., Kusnecov, A, **Pezzone, M.**, Berkun, J., & Rabin, B.S., Lactation alters the effects of conditioned stress on immune function, *American Journal of Physiology,* 272 (1997) R16-R25.

7. Turler, A., Moore, B.A., **Pezzone, M.A.**, Overhaus, M., Kalff, J.C., and Bauer, A.J. Colonic postoperative inflammatory ileus in the rat. *Annals of Surgery*, 236 (2002) 56-66.

8. **Pezzone, M.A.**, and Wald, A. Functional Bowel Disorders in Inflammatory Bowel Disease. *Gastroenterology Clinics of North America*, 31 (2002) 347-357.

9. **Pezzone, M.A.**, Watkins, S.C., Alber, S.M., King, W.E., de Groat, W.C., Chancellor, M.C., and Fraser, M.O. Identification of C-Kit-Positive Cells in the Ureter: The Interstitial Cells of Cajal of the Urinary Tract. *American Journal of Physiology* 284 (2003) 925-929.

10. Moore, B.A., Turler, A., **Pezzone, M.A.**, Dyer, K., Grandis, J., and Bauer, A.J. Tyrophostin AG126 inhibits the development of postoperative ileus induced by surgical manipulation of the murine colon. *American Journal of Physiology* 286 (2004) G214-G224.

11. Overhaus, M., Togel, S., **Pezzone, M.A.**, and Bauer, A.J. Mechanisms of polymicrobial sepsis induced-ileus. *American Journal of Physiology* 287 (2004) G685-G694.

12. **Pezzone, M.A.**, Liang, R., and Fraser, M.O. A Model of neural cross-talk and irritation in the pelvis: Implications for the overlap of chronic pelvic pain disorders. *Gastroenterology* 128 (2005) 1953-1964.

13. Ustinova, E.E., Fraser, M.O., and **Pezzone, M.A.** Colonic Irritation in the Rat Sensitizes Urinary Bladder Afferents to Mechanical and Chemical Stimuli: An Afferent Origin of Pelvic Organ Cross-Sensitization. *Am J Physiol Renal Physiol* 290:F1478-87, 2006.

14. Ustinova, E.E., Gutkin, D.W., and **Pezzone, M.A.** Sensitization of Pelvic Nerve Afferents and Mast Cell Infiltration in the Urinary Bladder Following Chronic Colonic Irritation is Mediated by Neuropeptides. *Am J Physiol Renal Physiol* 292:F123-130, 2007.

15. Christianson, J.A., Liang, R., Ustinova, E.E., Davis, B.M., Fraser, M.O., and **Pezzone, M.A.** Convergence of Bladder and Colon Sensory Innervation Occurs at the Primary Afferent Level. *Pain* 128:235-243, 2007.

16. Liang, R., Ustinova, E.E., Patnam, R., Fraser, M.O., Gutkin, D.W., and **Pezzone, M.A.** Enhanced Expression of Mast Cell Growth Factor and Mast Cell Activation in the Bladder Following the Resolution of Trinitrobenzesulfonic Acid (TNBS) Colitis in Female Rats. *Neurourology and Urodynamics* 26:887-893, 2007.

17. Sibille, E., Su, J., Leman, S., Le Guisquet, A.M., Ibarguen-Vargas, Y., Joeyen-Waldorf, J., Tseng, G., **Pezzone, M.A.**, Hen, R., Belzung, C. Lack of Serotonin1B Receptor Expression Leads to Age-related Motor Dysfunction, Early Onset of Brain Molecular Aging and Reduced Longevity. *Molecular Psychiatry* 12:1042-1056, 2007.

18. Bakdash, S., Lyons, J.M., Bastacky, S. I., **Pezzone, M.A.**, McGee, J.B., Schoen, R.E., Regueiro, M., Lee, K.K., and Bontempo, F.A. Management of Persistent Gastric Bleeding in a Patient with Glanzmann's Thrombasthenia. *American Journal of Hematology* 83:411-415, 2008.

19. Fitzgerald, J.J. and **Pezzone, M.A.** Role of Bowel Pathophysiology in Voiding Dysfunction. *Current Bladder Dysfunction Reports* 4:234-239, 2009.

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21. Fitzgerald, J.J., Ustinova, E.E., de Groat, W.C., and **Pezzone, M.A.** Evidence for the Role of Mast-cell Mediators and Their Targets in Bowel-bladder Cross-organ Sensitization. *Autonomic Neuroscience: Basic and Clinical,* 173:6-13, 2013.

22. Silos-Santiago, I., Hannig, G., Eutamene, H., Ustinova, E. E., Bernier, S. G., Ge, P., Jacobson, S., Jin, H., Reza, T., Shea, C., Kessler, M. M., Bryant, A. P., Kurtz, C. B., Bueno, L., **Pezzone, M. A.**,

and Currie, M. G. Visceral Pain: Unraveling a novel endogenous pathway through uroguanylin/guanylate cyclase-C receptor/cGMP activation. *Pain*, 154:1820-1830, 2013.

23. Mupparapu, S.K., and **Pezzone, M.A.** A Rare Case of Dual Cecae. ACG Case Rep J, 2:76, 2015.

24. Fitzgerald, J.J., Mupparapu, S.K., Ustinova, E.E., Watkins, S., de Groat, W.C., and **Pezzone, M.A.** Role of PAR-2 and Urothelium in Colon-Bladder Cross-Sensitization. Submitted.

Book Chapters/Reviews

1. Rabin, B.S., **Pezzone, M.A.**, Kusnecov, A.W., and Hoffman, G.E., Identification of stressor-activated areas in the central nervous system, In M. Ian Phillips and D. Evans (Eds.), *Methods in Neurosciences*, San Diego: Academic Press. Vol 24, 1995, pp 185-193.

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3. **Pezzone, M.A.,** and Wald, A. Irritable bowel syndrome. Physician's Practice Digest. Vol 11, 2001.

4. **Pezzone, M.A.**, de Groat, W.C., and Fraser, M.O. Evidence of Bidirectional, Cross-Sensitization of the Distal Colon and Lower Urinary Tract: A Possible Etiology of Concurrent Irritable Bowel Syndrome and Interstitial Cystitis. In: G. Holtmann and N.J. Talley (Eds.), *Gastrointestinal Inflammation and Disturbed Gut Function: The Challenge of New Concepts*. London: Kluwer Academic Publishers. pp. 17-28 (2003).

5. **Pezzone, M.A.** Neurophysiology of the Pelvis. In: P. Leppert and M. Turner (Eds.), *Vulvodynia: Toward Understanding a Pain Syndrome. Proceedings from the Workshop*. April 14-15, 2003. US Department of Health and Human Services, National Institutes of Health: Rockville, MD (2004).

6. **Pezzone, M.A.** Chronic Pelvic Pain and the Overlap of Chronic Pelvic Pain Disorders. Digestive Health Matters. International Foundation for Functional Gastrointestinal Disorders. Vol 15. No. 3. pp. 28-29 (2006).

7. Ustinova, E.E., Fraser, M.O., and **Pezzone, M.A**. Pelvic Visceral Pain and Cross-Sensitization Among Organs. In D. Bjorling (Ed.), *Visceral Pain*. Kerala, India: Transworld Research Network. Pp. 107-119 (2010).

8. **Pezzone, M.A.** Irritable Bowel Syndrome. GI Rounds Online. Girounds.pitt.edu. 12/1/2010.

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1. Cunnick, J.E, Lysle, D.T., Fraser, M.O., **Pezzone, M.A.**, and Rabin, B.S., Inhibition of

sympathetic output attenuates shock-induced suppression of mitogenic activity, *Society for Neuroscience Abstracts*, 16 (1990) 1211. St. Louis, MO.

2. Fraser, M.O., Hoffman, G.E., Lysle, D.T., Cunnick, J.E., **Pezzone, M.A.**, Kucinski, B.J., and Rabin, B.S., Brain c-Fos immunoreactivity induced by conditioned and unconditioned aversive stimuli, *Society for Neuroscience Abstracts*, 16 (1990) 1199. St. Louis, MO.

3. Rabin, B.S., **Pezzone, M.A.**, Lee, W.-S., Hoffman, G.E., and Pezzone, K.M., Stressor-induced c-Fos expression in brain stem loci of the rat: a correlation of neuronal activation with immune alteration. *Journal of Neuroimmunology*, S1 (1991) 89. Third International Congress on Neuroimmunology. Jerusalem, Israel.

4. **Pezzone, M.A.**, Lee, W.-S., Hoffman, G.E., Pezzone, K.M., and Rabin, B.S., Central catecholaminergic pathways implicated in stressor-induced immune alteration of splenic lymphocytes, *Research Perspectives in Psychoneuroimmunology III*, Columbus, OH, 1991.

5. **Pezzone, M.A.**, Lee, W.-S., Hoffman, G.E., Pezzone, K.M., and Rabin, B.S., Stressor-induced c-Fos expression in brain stem loci of the rat: a correlation of neuronal activation with immune alteration, *Society for Neuroscience Abstracts*, 17 (1991) 1200. New Orleans, LA.

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